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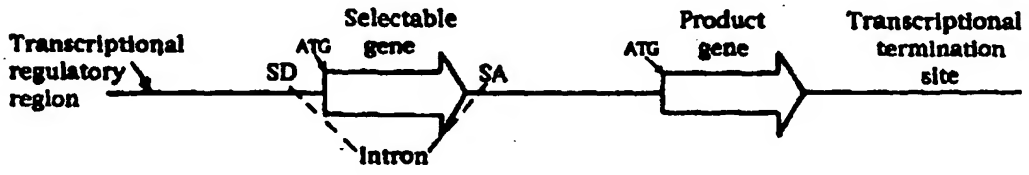

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5' end of the transcribed region and a selectable gene at the 3' end. Because translation of the selectable gene at the 3' end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., 264(24): 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (▲GT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [▲GT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ▲GT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	Na^+K^+-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5' end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3' end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., 10:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., 9:329 (1989); Gatermann *et al.*, Mol. Cell Biol., 9:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., 68:90 (1979); Caruthers *et al.*, Meth. Enzymol., 154:287 (1985); Froehler *et al.*, Nuc. Acids Res., 14:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerasechain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton *et al.*, Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen *et al.*, Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica *et al.*, Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR$^-$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR$^-$ cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT 1000

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA 3800

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

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GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG 6800

CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT 6900

CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

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AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC 1600

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT 3150

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

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GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA 6800

CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

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TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC 4300

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AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

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CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

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AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

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GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

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CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 2100

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT 2150

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GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG 2250

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ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2350

GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC 2400

GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 2450

CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC 2500

GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG 2550

CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG 2600

AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT 2650

ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA 2700

ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC 2750

CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC 2800

TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT 2850

TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA 2900

GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT 2950

GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG 3000

TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC 3050

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CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT 3150

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CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

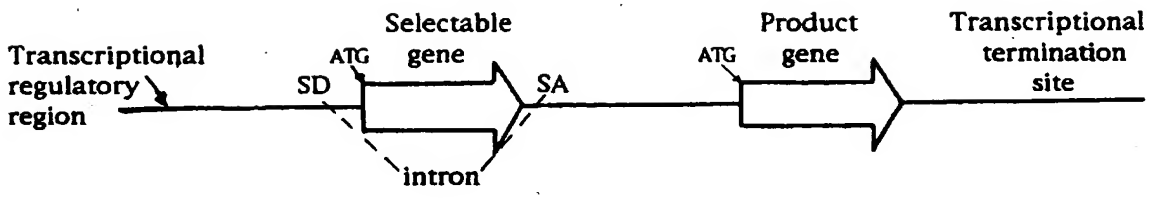
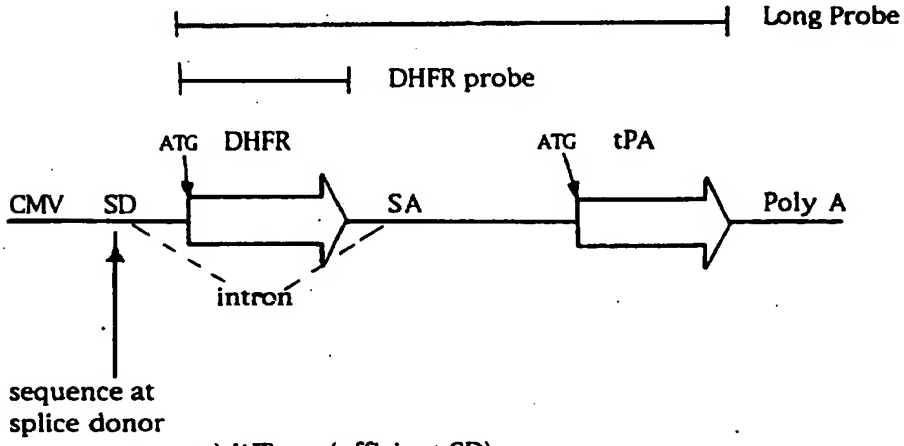
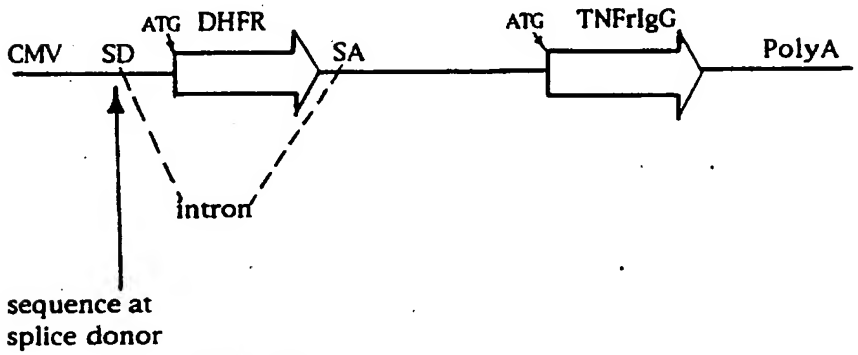


FIG. 1B



a) WT ras (efficient SD)
b) MUTANT ras (less efficient SD)
c) ▲GT (inefficient SD)

FIG. 1C



a) WT ras
b) MUTANT ras
c) ▲GT

FIG. 1D

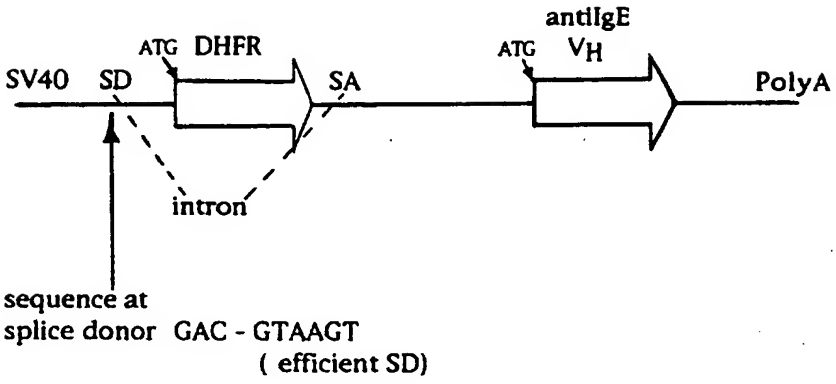

antiIgE
ATG DHFR
ATG V_H
SV40 SD
SA
PolyA
intron
sequence at
splice donor GAC - GTAAGT
(efficient SD)

FIG. 2

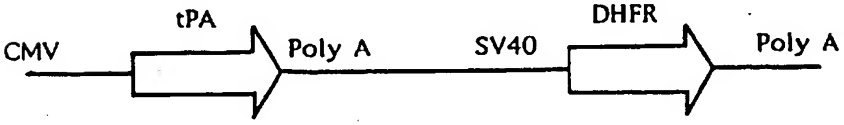

tPA
DHFR
CMV
Poly A
SV40
Poly A

FIG. 3A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                     thaI
       bsiHKAI                                                                                     fnuDII/mvnI
       bmyI                       rmaI    tru9I                                                    bstUI
       banII                      maeI    mseI                                                     bsh1236I
     taqI                        speI    aseI/asnI/vspI                           bslI            aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                maeII
                sau96I                                   hinlI/acyI
                haeIII/palI     aciI                     ahaII/bsaHI
                asuI  apyI[dcm+]                aciI     aatII          maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                      maeII
      hinlI/acyI                                                                                 hinlI/acyI
      ahaII/bsaHI                                     rsaI                        rsaI           ahaII/bsaHI
      aatII                              bglI         csp6I            ndeI       csp6I          aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                      nlaIII
         bglI  dsaV                                                                            styI
        sau96I bstNI            rsaI                                         maeII                  ncoI
        haeIII/palI             csp6I                              rsaI    snaBI                    dsaI hphI  aciI
        asuI   apyI(dcm+)   bsrI  nlaIII                           csp6I   bsaAI                    bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
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FIG. 3B

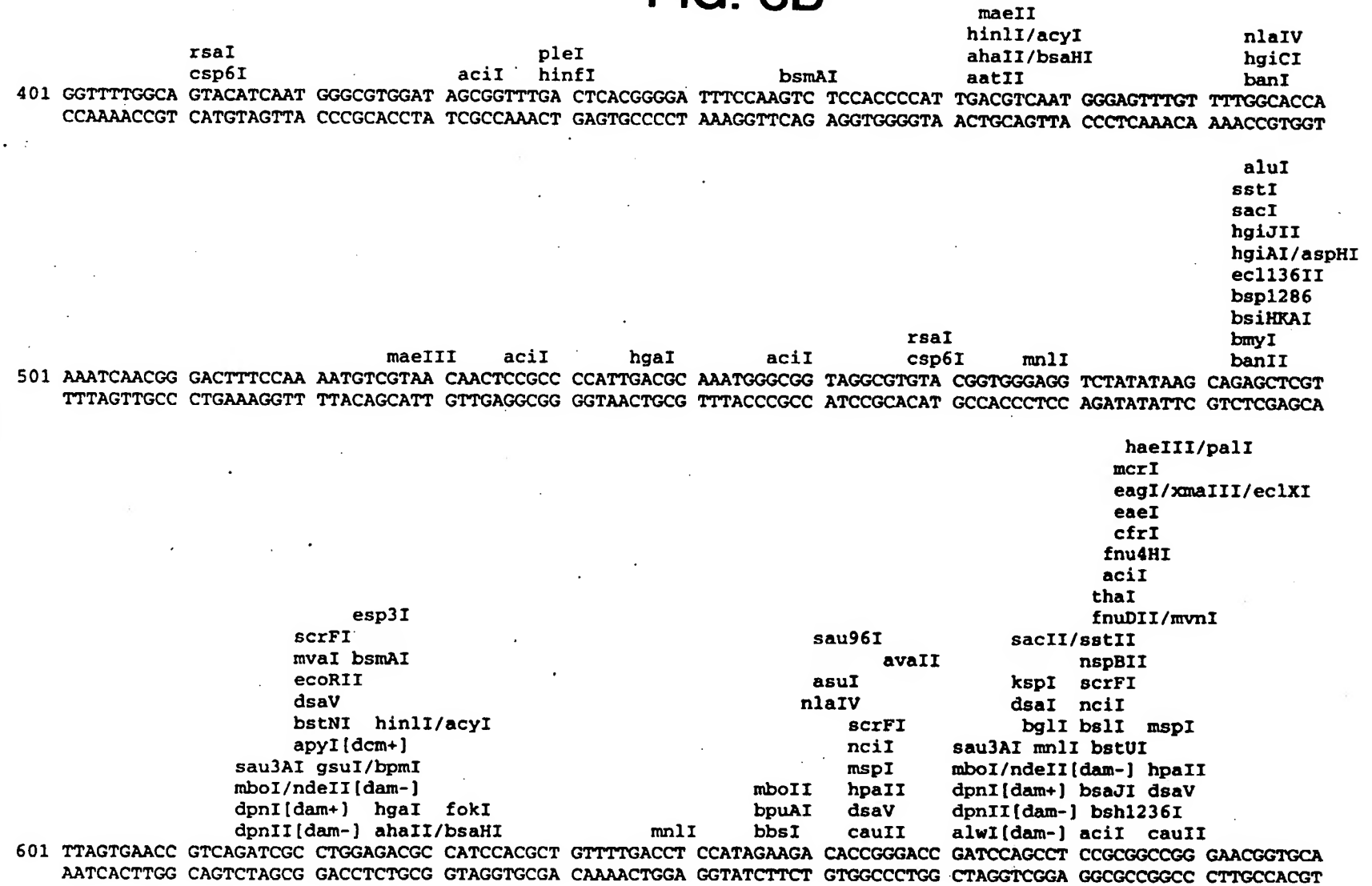

FIG. 3C

```
               tfiI
            aciI
           thaI hinfI
           fnuDII/mvnI
           bstUI
           bsh1236I                                  aciI
                                                   rsaI                                          fnu4HI
                                                   csp6I     scfI                                bbvI
                                                                          mnlI                   nspBII         taqI
                                                                                                 aciI       nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                                 thaI
                                                                                                 fnuDII/mvnI
                                                                                                 bstUI
                                                                                                 bsh1236I
                                                                                               mluI
                                                                                   bsrBI       aflIII       rsaI
                        pflMI                             bsmAI                    aciI       xmnI          csp6I
     sfaNI              bslI                              bsaI                  mnlI   ddeI   asp700       scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
         eco57I                                                   bstNI
        mboII                       tfiI                          apyI[dcm+]                 tfiI          tru9I          tru9I
       earI/ksp632I                 hinfI                                              mboII  taqI    mseI           mseI
      mnlI                        alwNI        hphI            sexAI           ddeI           hinfI   ahaIII/draI     aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                             aluI
                                             sstI
                                             sacI
                                             hgiJII
                                             hgiAI/aspHI
                                             ecl136II
                                             bsp1286
                                             bsiHKAI
                                             bmyI                                            tru9I
                                             banII                                 sfaNI     mseI           mspI
           ddeI                    bslI     mnlI                   bstXI       fokI        aflII/bfrI      hpaII
                                                                                                            bsaWI
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     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

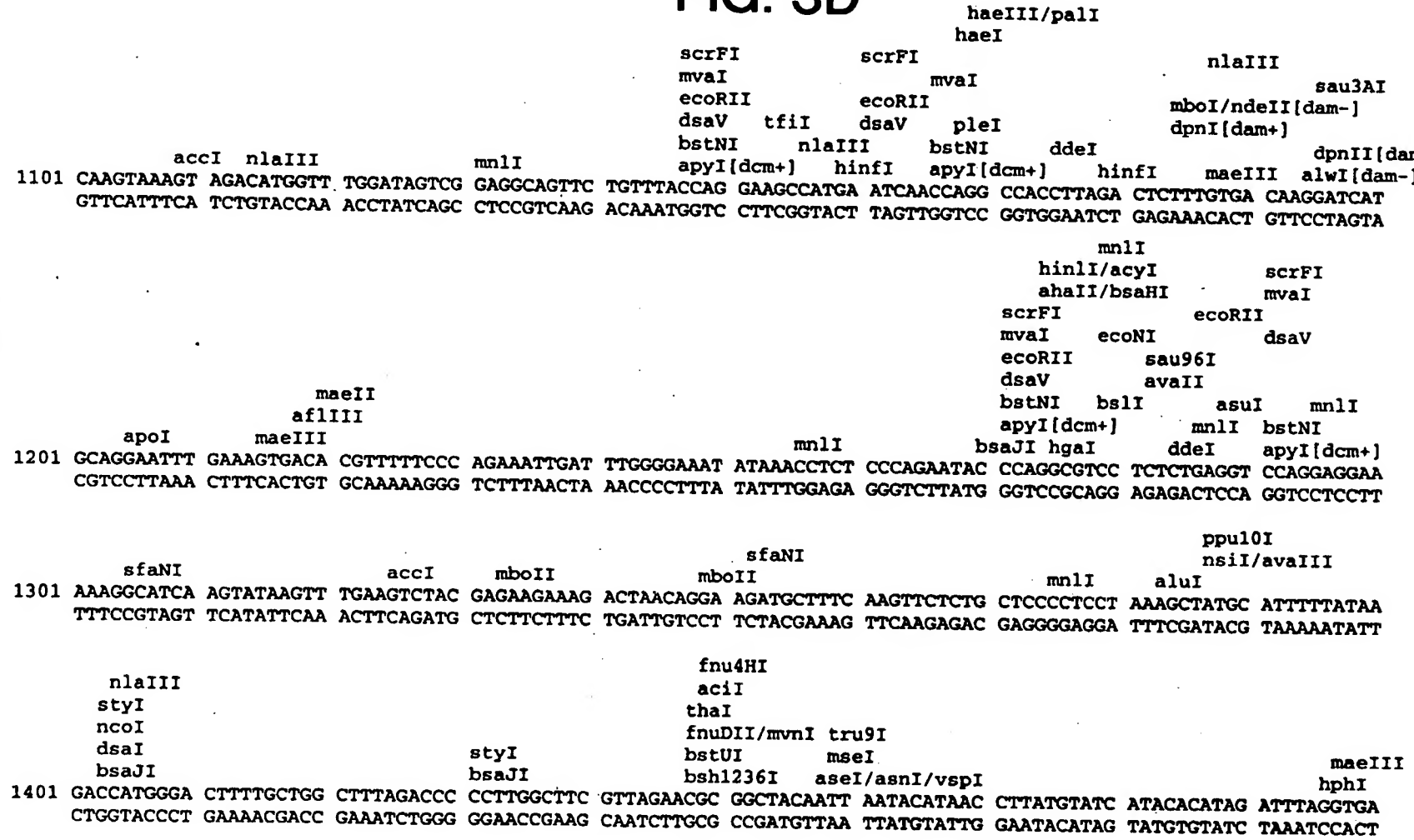

FIG. 3E

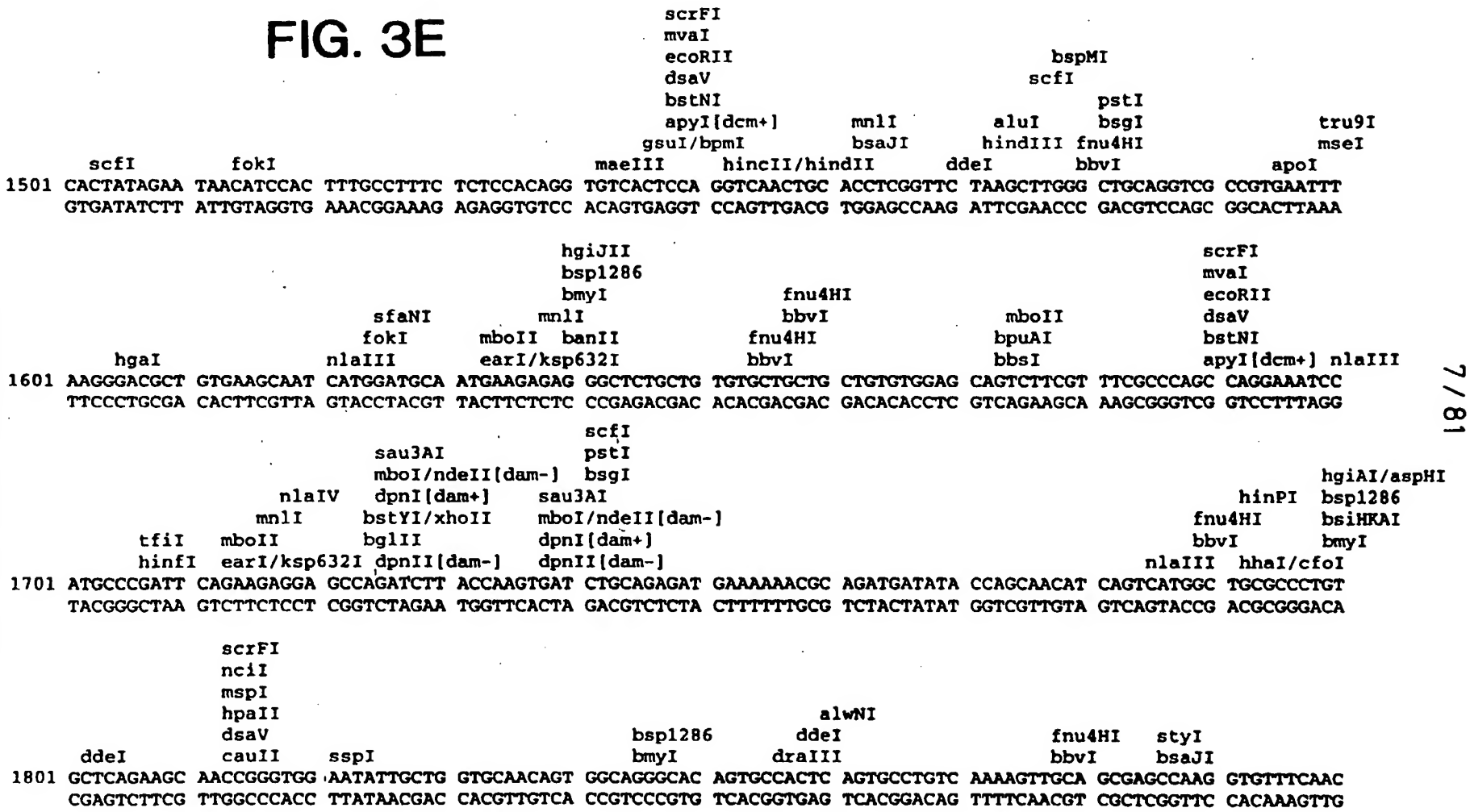

FIG. 3F

```
                                                                                                                         pmlI
                                                                                                                          eco72I
                                                                                                                      sau96I
                                                                                                                      asuI maeII
                                                                                                                  scrFI
        bspMI                                                                                                     mvaI haeIII/palI
     nlaIV          sau96I                                                                                        ecoRII
     hgiCI          haeIII/palI                                                                                   dsaV
     banI           asuI   rsaI                          bsp1286                                                  bstNI   bsaAI
    bsp1286        eco0109I/draII                        bmyI                                                     bsaJI   bbrPI
    bmyI          alwNI    csp6I ddeI                 bsrI      bcgI                                              apyI[dcm+]
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

                scrFI                                          hinPI
                mvaI                                                hhaI/cfoI
                ecoRII                                        nlaIV   hgiAI/aspHI
                dsaV                                          narI    bsp1286               thaI
                bstNI                   pmlI  hgiAI/aspHI           kasI     bsiHKAI              fnuDII/mvnI
                bsaJI                   eco72I                      hinlI/acyI                    bstUI  sau96I
            sau96I                 bsaAI bsp1286              hgiCI   bmyI                  hinPI haeIII/palI
            avaII                  bbrPI bsiHKAI              haeII   apaLI/snoI            hhaI/cfoI
            asuI     sfaNI    scfI  bsp1286    bmyI    aciI       banI     alw44I/snoI        fnu4HI      asuI      bslI
          mnlI  apyI[dcm+] aluI    bmyI maeII       nspBII        ahaII/bsaHI       bsrI      bbvI bsh1236I        bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                               scrFI
                                               mvaI
                                               ecoRII
                                               dsaV                                           pleI       scrFI
                                               bstNI                                              bsmAI        mvaI
                                               bsaJI                                            taqI[dam-]     ecoRII
                               hinlI/acyI      apyI[dcm+]                                     sau3AI hinfI     dsaV
                aciI  haeIII/palI            haeIII/palI              scfI                    mboI/ndeII[dam-] bstNI
      nspBII      haeI  hgaI bstXI          sau96I             pstI              dpnI[dam+]          apyI[dcm+]      tru9I
     scfI aciI     mnlI   ahaII/bsaHI        asuI       nlaIV           bsgI         dpnII[dam-]        bsaJI        maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
```

FIG. 3G

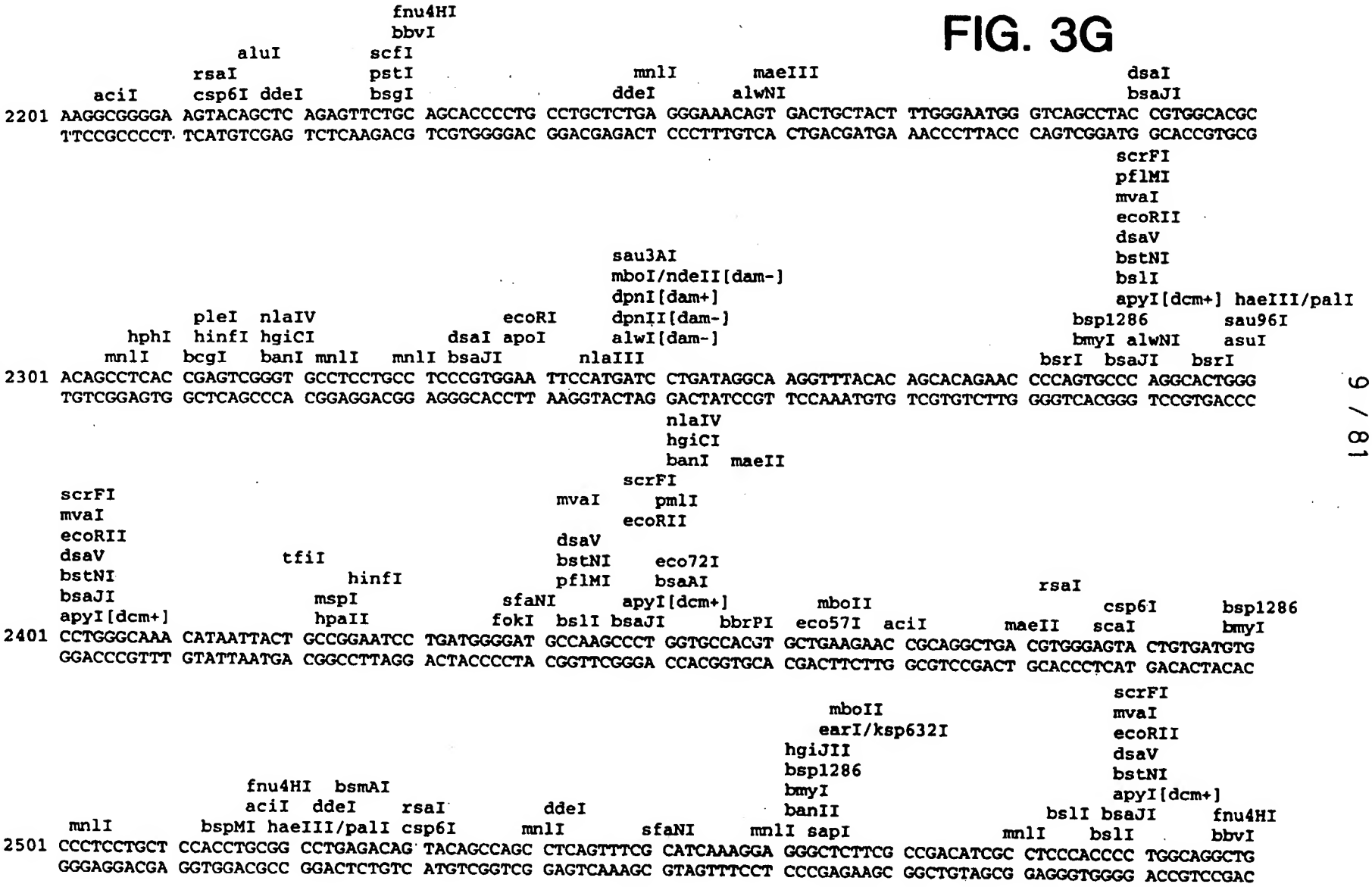
fnu4HI
bbvI
aluI
scfI
rsaI
pstI
mnlI
maeIII
dsaI
aciI
csp6I ddeI
bsgI
ddeI
alwNI
bsaJI
2201 AAGGCGGGGA AGTACAGCTC AGAGTTCTGC AGCACCCCTG CCTGCTCTGA GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC
TTCCGCCCCT TCATGTCGAG TCTCAAGACG TCGTGGGGAC GGACGAGACT CCCTTTGTCA CTGACGATGA AACCCTTACC CAGTCGGATG GCACCGTGCG
scrFI
pflMI
mvaI
ecoRII
dsaV
sau3AI
bstNI
mboI/ndeII[dam-]
bslI
dpnI[dam+]
apyI[dcm+] haeIII/palI
pleI nlaIV
ecoRI
dpnII[dam-]
bsp1286
sau96I
hphI hinfI hgiCI
dsaI apoI
alwI[dam-]
bmyI alwNI
asuI
mnlI bcgI banI mnlI mnlI bsaJI
nlaIII
bsrI bsaJI bsrI
2301 ACAGCCTCAC CGAGTCGGGT GCCTCCTGCC TCCCGTGGAA TTCCATGATC CTGATAGGCA AGGTTTACAC AGCACAGAAC CCCAGTGCCC AGGCACTGGG
TGTCGGAGTG GCTCAGCCCA CGGAGGACGG AGGGCACCTT AAGGTACTAG GACTATCCGT TCCAAATGTG TCGTGTCTTG GGGTCACGGG TCCGTGACCC
nlaIV
hgiCI
banI maeII
scrFI
scrFI
mvaI
pmlI
mvaI
ecoRII
ecoRII
dsaV
dsaV
tfiI
bstNI
eco72I
bstNI
hinfI
pflMI
bsaAI
bsaJI
rsaI
mspI
sfaNI
apyI[dcm+]
mboII
csp6I
bsp1286
apyI[dcm+]
hpaII
fokI bslI bsaJI
bbrPI eco57I aciI
maeII scaI
bmyI
2401 CCTGGGCAAA CATAATTACT GCCGGAATCC TGATGGGGAT GCCAAGCCCT GGTGCCACGT GCTGAAGAAC CGCAGGCTGA CGTGGGAGTA CTGTGATGTG
GGACCCGTTT GTATTAATGA CGGCCTTAGG ACTACCCCTA CGGTTCGGGA CCACGGTGCA CGACTTCTTG GCGTCCGACT GCACCCTCAT GACACTACAC
scrFI
mboII
mvaI
earI/ksp632I
ecoRII
hgiJII
dsaV
bsp1286
bstNI
fnu4HI bsmAI
bmyI
apyI[dcm+]
aciI ddeI rsaI
ddeI
banII
bslI bsaJI fnu4HI
mnlI
bspMI haeIII/palI csp6I
mnlI
sfaNI
mnlI sapI
mnlI bslI bbvI
2501 CCCTCCTGCT CCACCTGCGG CCTGAGACAG TACAGCCAGC CTCAGTTTCG CATCAAAGGA GGGCTCTTCG CCGACATCGC CTCCCACCCC TGGCAGGCTG
GGGAGGACGA GGTGGACGCC GGACTCTGTC ATGTCGGTCG GAGTCAAAGC GTAGTTTCCT CCCGAGAAGC GGCTGTAGCG GAGGGTGGGG ACCGTCCGAC

FIG. 3H

```
                                  mspI
                                  hpaII
                                  bslI
                                  scrFI                                                                             scrFI
                                  nciI      nlaIV                                                                   mvaI
                                  dsaV     aciI                                                                     ecoRII
                                  cauII   bsrBI                          aluI        tfiI        aciI              dsaV
            bslI       mnlI                          bslI aciI           alwNI       hinfI       fnu4HI            bstNI
                                                                                                                    apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                                    scrFI
                                                                    mvaI
                                                                    ecoRII
                                                                    dsaV
                                                                    bstNI
                                                                    apyI[dcm+]
                                                         scrFI     bslI
                                     sau3AI              nciI      bsaJI
                                     mboI/ndeII[dam-]    mspI    sau96I
                                     dpnI[dam+]          hpaII   nlaIV
                                     dpnII[dam-]         dsaV    avaII        mnlI
      mnlI      aciI      bslI     hphI[dam-]            cauII   asuI      mnlI          apoI        taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                         scfI                                 scrFI
                                                         pstI                                 mvaI
                                                        fnu4HI                                ecoRII
                                                        bbvI pvuII                            dsaV
                                                     fnu4HI fnu4HI                            bstNI              aciI
                                                     bbvI    bbvI                          bsp1286      fnu4HI   sau96I
            ecoRI                                  hinPI bsgI aluI        tfiI        nspBII   bsaJI    bbvI     avaII
            apoI taqI                              hhaI/cfoI nspBII       hinfI       aciI bmyI apyI[dcm+]       asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

```
                                                                          aluI
                                                                          sstI
                                          pvuII                           sacI
                      mspI       scfI                                     hgiJII
                      hpaII      pstI   fnu4HI                            hgiAI/aspHI
                      scrFI    bspMI    bbvI                              ecl136II                  haeIII/palI
                      nciI   sau96I nspBII                                bsp1286                    stuI
                      dsaV   avaII fnu4HI       bsrI                      bsiHKAI                    haeI
                      cauII  asuI  bbvI     mspI                          bmyI     mspI             mnlI
      draIII    bslI bsaJI aciI  bsgI aluI hpaII                          banII    hpaII        nlaIII      bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                   fnu4HI
                                                 nspBII
       fnu4HI                                    aciI                                                 nlaIII
       aciI                             rsaI fokI  fnu4HI                  tru9I        hphI        nspI
      bsrBI eco57I mnlI  nlaIII         csp6I    alwNI bbvI                msel         maeIII      nspHI       gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                        sau96I
                        nlaIV
                        haeIII/palI
                        asuI
                       sau96I
                       nlaIV scrFI                                             scrFI
                       hgiJII                                                      mvaI
                       eco0109I/draII                      scrFI               ecoRII
                       bsp1286                             mvaI                    dsaV
                       bsp120I                             ecoRII              bstNI
                       bmyI   mvaI                         dsaV            sau96I
                       banII ecoRII              bstNI                     nlaIV                        aciI
                     aciI    dsaV                bsaJI                     haeIII/palI                 fnu4HI
                  fnu4HI     bstNI             hinlI/acyI       tfiI          asuI apyI[dcm+]            haeIII/palI
                  aciI asuI  apyI[dcm+]        hgaI       apyI[dcm+]        eco0109I/draII              eaeI
      bsmAI     bsrBI   apaI bsaJI             ahaII/bsaHI      hinfI       mnlI  bsaJI                 cfrI   nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
```

FIG. 3J

```
                                                       scrFI
                                                       nciI
                            scrFI                mspI
                            mvaI                       hpaII
                            ecoRII               dsaV
                            dsaV                 cauII
                            bstNI                     xmaI/pspAI
                            bsaJI                     smaI
                            apyI[dcm+]                scrFI
                       nlaIV                          nciI
                       eco0109I/draII           dsaV
                   aluI sau96I                  cauII     rsaI  styI maeIII                tfiI        nspI
                  pvuII asuI                          bsaJI     csp6I bsaJI            rmaI     hinfI      nspHI
      sfaNI       nspBII haeIII/palI            fokI  avaI     bsp1407I  bstEII        maeI bsrI         maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                            mboII
               scrFI                            aciI earI/ksp632I
               mvaI                               sau3AI mnlI
               ecoRII                       mboI/ndeII[dam-]
               dsaV                         dpnI[dam+]           mboII
               bstNI           mnlI               dpnII[dam-]    eco57I
        bslI   apyI[dcm+]   pleI                  alwI[dam-]    mboII  bpuAI          hinPI
    mcrI maeIII             hinfI                bstYI/xhoII  mboII    bbsI           hhaI/cfoI      scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                       bsmAI              mboII
     gsuI/bpmI        aciI     aciI    bsaI  scfI     mnlI earI/ksp632I                                   tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                  scrFI
                                                               scrFI       mvaI
                                                               mvaI bsmI          ecoRII
                                                               ecoRII      dsaV
                                                               dsaV        bstNI
                                      mboII                    bstNI              bsaJI
                                      bpuAI   bsmI     rmaI   gsuI/bpmI   mnlI   bsaJI                  ecoRI   taqI
                                      bbsI nlaIII      maeI mnlI apyI[dcm+]   mnlI apyI[dcm+]           apoI   claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
```

FIG. 3K

```
                styI
              aciI
             fnu4HI   sau96I
             bglI nlaIII
            sfiI ncoI haeIII/palI
            haeIII/palI                    aluI
           eaeI  dsaI asuI               fnu4HI
           cfrI  bsaJI                   bbvI          maeIII              sfaNI  apoI                                  bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                                  sau3AI
                                                                  mboI/ndeII[dam-]
                                                                  dpnI[dam+]
                                                                  dpnII[dam-]
                                                                 pvuI/bspCI
                                                                 mcrI
                                                                taqI[dam-]  tru9I
                                                              claI/bsp106[dam-]              styI
                                                             sau3AI          mseI       fnu4HI    haeI
                                                             mboI/ndeII[dam-]           bbvI  ncoI
                                                             dpnI[dam+] xmnI        hinPI    dsaI haeIII/palI
           rmaI                                              dpnII[dam-]    aseI/asnI/vspI    bsaJI
           maeI                                        nlaIII alwI[dam-]  asp700      hhaI/cfoI nlaIII           mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                         rsaI                                                                        nlaIV
                         csp6I                                                                     scrFI
                        nlaIV                                                                      mvaI
                        kpnI                                                                       ecoRII
                        hgiCI                                                                      dsaV
                        banI                              aluI                                     bstNI
                        asp718     mnlI                   pvuII                                    apyI[dcm+]
           mnlI         acc65I   ddeI aciI               nspBII                                   bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                                                                      nlaIV
               sfaNI                     scrFI       scrFI                                  sfaNI
             ppu10I                      mvaI              mvaI                                       ppu10I
             nsiI/avaIII                 ecoRII      ecoRII                               nsiI/avaIII
            nlaIII                       dsaV        dsaV                                nlaIII
           sphI                          bstNI             bstNI                                  sphI
           nspI                          apyI[dcm+]        apyI[dcm+]                             nspI
           nspHI                        sexAI             bsaJI                                   nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                           nlaIII
                                                                                          styI
                                                                                          ncoI
                                             aciI                                   bslI  dsaI
               aciI        aciI  fokI                    aciI bsrI  aciI            aciI  bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                                   rmaI
                                                                                  styI
               aciI                                                               bsaJI
              fnu4HI                                                              blnI
             sfiI                                                                 avrII
             haeIII/palI                                                        haeIII/palI        tru9I
         bsaJI bglI          ddeI                                              stuI              hpaI
       haeIII/palI mnlI haeIII/palI                          mnlI              haeI              hincII/hindII
     mnlI    mnlI   bsaJI  mnlI  aluI                    mnlI                 mnlI maeI        aluI mseI  aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                          scrFI
                                          mvaI
                                          ecoRII
      haeIII/palI                         dsaV
     eaeI                                 bstNI                                               aluI
     cfrI                     bsrI        apyI[dcm+]       tru9I       fnu4HI                pvuII
    bsrI             maeII maeIII        bsaJI   maeIII    mseI        bbvI  fokI             nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

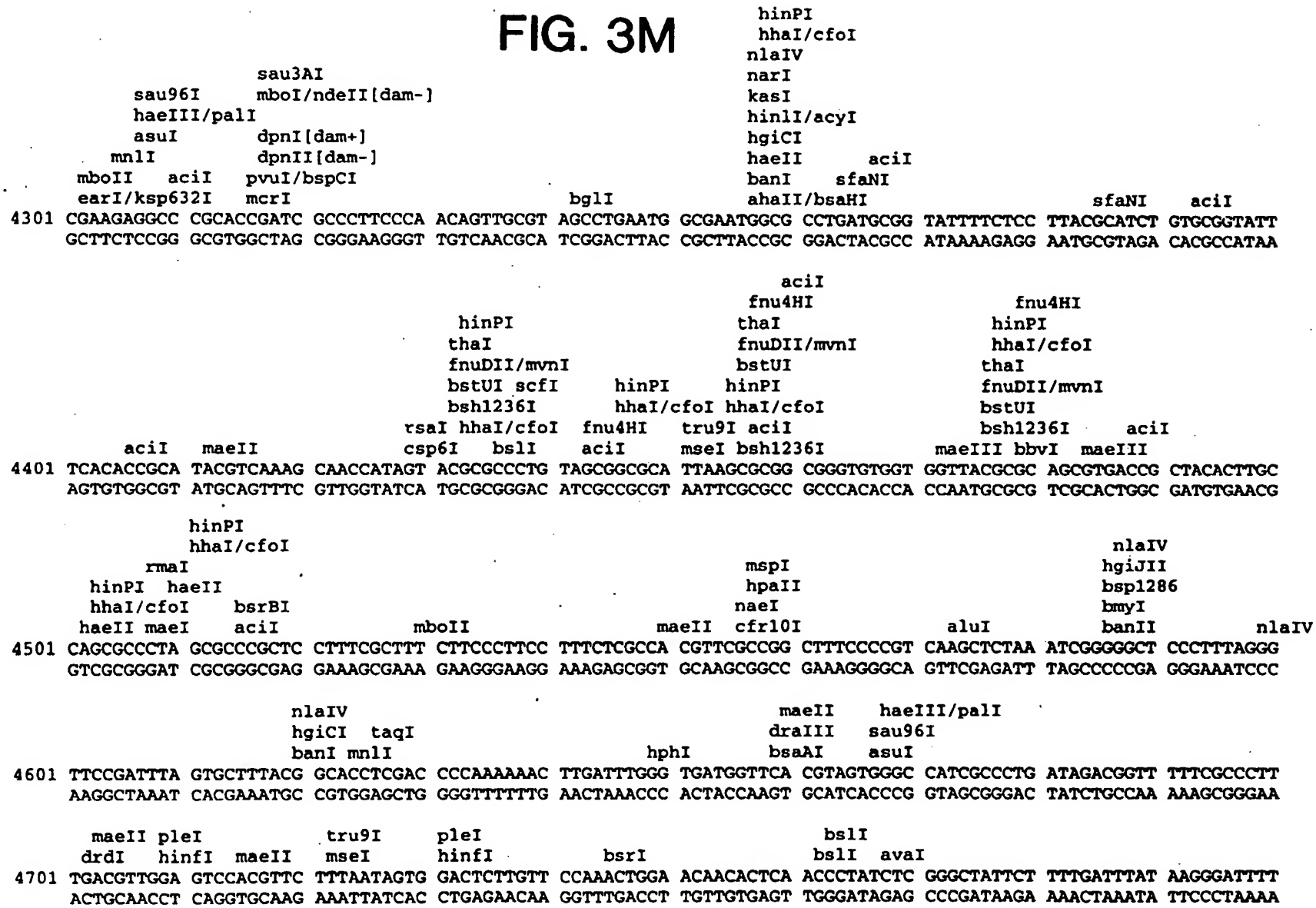
hinPI
hhaI/cfoI
nlaIV
narI
kasI
hinlI/acyI
hgiCI
haeII aciI
banI sfaNI
ahaII/bsaHI sfaNI aciI
sau3AI
sau96I mboI/ndeII[dam-]
haeIII/palI
asuI dpnI[dam+]
mnlI dpnII[dam-]
mboII aciI pvuI/bspCI
earI/ksp632I mcrI bglI
4301 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT
GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA
aciI
fnu4HI
thaI
fnuDII/mvnI
bstUI
hinPI
thaI
fnuDII/mvnI
bstUI scfI
bsh1236I
hinPI
hhaI/cfoI
rsaI hhaI/cfoI
csp6I
fnu4HI
bslI
aciI
tru9I aciI
mseI bsh1236I
fnu4HI
hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
bstUI
bsh1236I aciI
maeIII bbvI maeIII
aciI maeII
4401 TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC
AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG
hinPI
hhaI/cfoI
rmaI
hinPI haeII
hhaI/cfoI bsrBI
haeII maeI aciI
mboII
mspI
hpaII
naeI
maeII cfr10I
aluI
nlaIV
hgiJII
bsp1286
bmyI
banII nlaIV
4501 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC
nlaIV
hgiCI taqI
banI mnlI
hphI
maeII
draIII
bsaAI
haeIII/palI
sau96I
asuI
4601 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT
AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA
maeII pleI
drdI hinfI maeII
tru9I
mseI
pleI
hinfI
bsrI
bslI
bslI avaI
4701 TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT
ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA

FIG. 3N

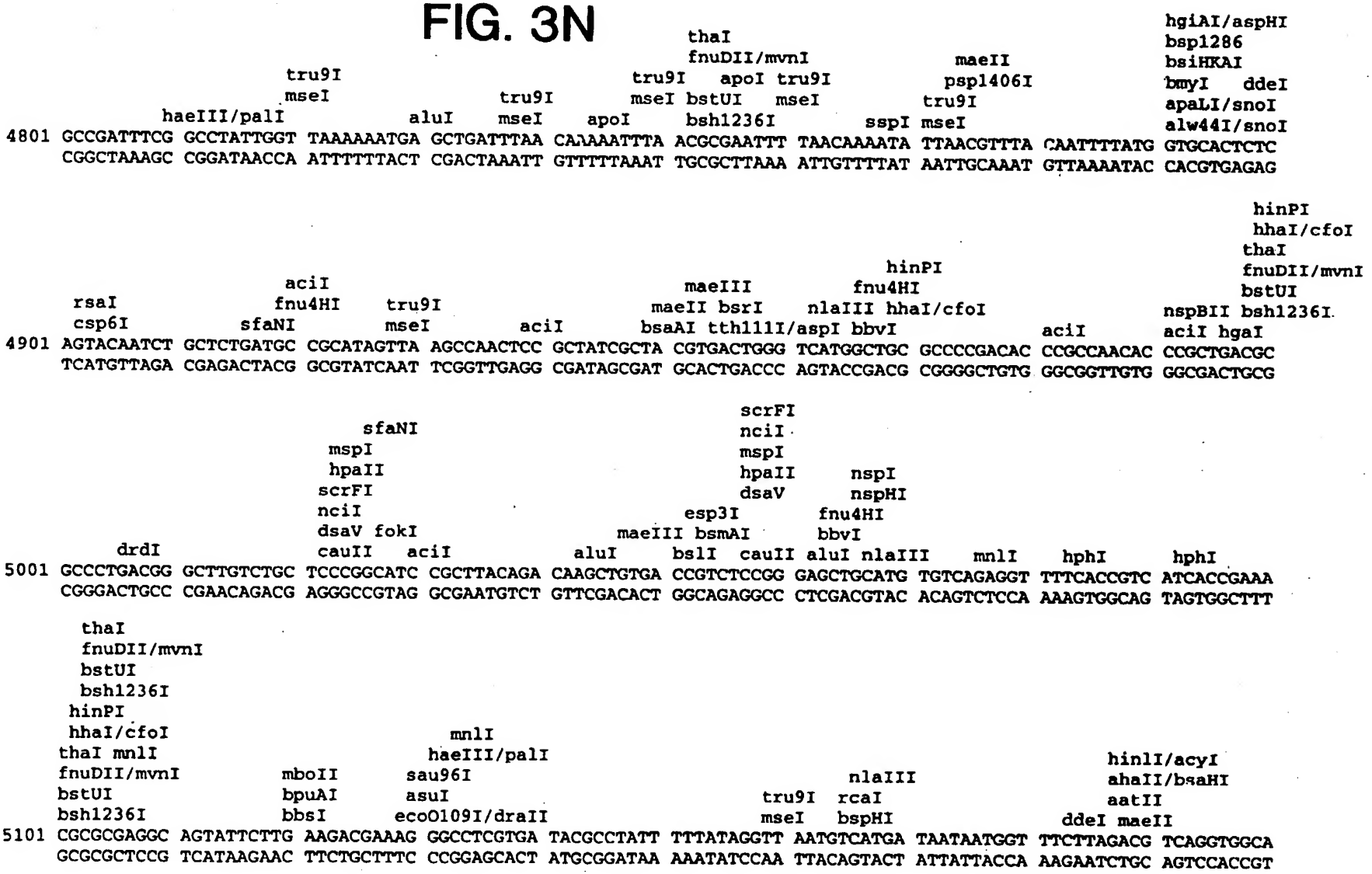
thaI
fnuDII/mvnI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI ddeI
apaLI/snoI
alw44I/snoI
tru9I
mseI
haeIII/palI
aluI
apoI
bsh1236I
maeII
psp1406I
sspI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG
hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
bstUI
nspBII bsh1236I
aciI
hgaI
rsaI
csp6I
aciI
fnu4HI
sfaNI
tru9I
mseI
maeIII
maeII bsrI
bsaAI tth111I/aspI
hinPI
fnu4HI
nlaIII hhaI/cfoI
bbvI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG
scrFI
nciI
sfaNI
mspI
hpaII
mspI
hpaII
nspI
scrFI
dsaV
nspHI
nciI
esp3I
fnu4HI
dsaV fokI
maeIII bsmAI
bbvI
drdI
cauII aciI
aluI
bslI cauII aluI nlaIII
mnlI
hphI
hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT
thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
mnlI
thaI mnlI
haeIII/palI
hinlI/acyI
fnuDII/mvnI
mboII
sau96I
nlaIII
ahaII/bsaHI
bstUI
bpuAI
asuI
tru9I rcaI
aatII
bsh1236I
bbsI
eco0109I/draII
mseI bspHI
ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT

FIG. 3O

```
                        nlaIV
                       aciI
                      thaI
                      fnuDII/mvnI
                      bstUI                                                    rcaI
                      bsh1236I                                                 bspHI
                     hinPI                                                  bsrBI    bsmAI
                     hhaI/cfoI                                              aciI  nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
     GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT

                 mboII                                                        fnu4HI
      sspI       earI/ksp632I                                                 aciI                              hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
     TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

                                        hgiAI/aspHI
                                        bsp1286
                           sau3AI       bsiHKAI                    sau3AI   nspBII     sau3AI
                           mboI/ndeII[dam-]                        mboI/ndeII[dam-]   mboI/ndeII[dam-]
                           dpnI[dam+]   bmyI                       dpnI[dam+]         dpnI[dam+]
                           dpnII[dam-]                            bstYI/xhoII         dpnII[dam-]
                       eco57I           apaLI/snoI            bsrI dpnII[dam-]        alwI[dam-]
      hphI          sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI    alwI[dam-]  aciI  bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
     TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG

                                                                          scrFI
                                                    aciI                  nciI
                                                   thaI                   mspI
        maeII                                      fnuDII/mvnI            hpaII
       psp1406I          hgiAI/aspHI               bstUI                  dsaV
       xmnI              bsp1286  tru9I            bsh1236I          hinlI/acyI
       asp700            bsiHKAI  mseI             hinPI             hgaI cauII                    aciI
     mboII               bmyI   ahaIII/draI        hhaI/cfoI         ahaII/bsaHI     bcgI  mcrI  fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
     GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA

                           rsaI
                           csp6I   bsrI                                                    fnu4HI
          ddeI             scaI  hphI  maeIII      sfaNI     fokI   nlaIII                bbvI        nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
     TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG
```

FIG. 3P


sau96I
avaII
sau3AI asuI
nlaIII
haeIII/palI
mboI/ndeII[dam-]
sau3AI maeIII
eaeI
dpnI[dam+]
mboI/ndeII[dam-]
cfrI
dpnII[dam-]
dpnI[dam+]
fnu4HI
pvuI/bspCI
dpnII[dam-]
aciI
mcrI mnlI
aluI aciI
nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC
TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG
hinPI
mspI
hhaI/cfoI
sau3AI nlaIV
mstI
mboI/ndeII[dam-] aluI
aviII/fspI bsrI
dpnI[dam+] hpaII
fnu4HI maeII tru9I
dpnII[dam-] bsaWI
maeIII sfaNI bbvI psp1406I mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG
mspI
hpaII
bglI
scrFI
sau96I
aluI nciI tru9I fokI sau96I haeIII/palI
rmaI dsaV mseI bsrI aciI avaII hinPI asuI mspI
maeI cauII aseI/asnI/vspI mnlI asuI hhaI/cfoI hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG
mspI thaI
hpaII fnuDII/mvnI haeIII/palI
cfr10I bstUI sau96I
nlaIV hphI bsmAI aciI fnu4HI nlaIV
gsuI/bpmI bsaI bsh1236I bbvI bsrI asuI mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA
ddeI
sau3AI nlaIV
pleI
mboI/ndeII[dam-]
hinfI
dpnI[dam+] hgiCI tru9I
eam1105I fokI dpnII[dam-] banI mnlI mseI maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT

FIG. 3Q

```
                                                                   rmaI       sau3AI
                                                            sau3AI hphI      mboI/ndeII[dam-]
                                                            mboI/ndeII[dam-]
                                                            dpnI[dam+]       dpnI[dam+]
                                                            dpnII[dam+]      dpnII[dam-]
                                    tru9I                tru9I bstYI/xhoII   alwI[dam-]         nlaIII              maeII
                                    mseI           tru9I mseI  alwI[dam-]  bstYI/xhoII         rcaI              tru9I
                                   ahaIII/draI     mseI ahaIII/draI maeI  mboII[dam-]          bspHI             mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                          sau3AI
                                                          mboI/ndeII[dam-]
                                                          dpnI[dam+]  sau3AI
                                                          dpnII[dam-] mboI/ndeII[dam-] thaI
                                                         bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                                  sau3AI  alwI[dam-]  dpnII[dam-]      bstUI
                                                  mboI/ndeII[dam-]    alwI[dam-]       bsh1236I
                                hgaI              dpnI[dam+] mboII[dam-]              hinPI      fnu4HI
                            ddeI                  dpnII[dam-]       bstYI/xhoII        hhaI/cfoI  bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                             sau3AI
                                             mboI/ndeII[dam-]
                                             dpnI[dam+]
                                             dpnII[dam-]
                                            alwI[dam-]
                             aciI           mspI                                    bsrI               hinPI
                  aciI    nspBII            hpaII       aluI                     maeIII    eco57I     hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                          fnu4HI
                                                                                                  alwNI   bbvI
           rmaI                 haeIII/palI                                                     bsrI  fnu4HI
           maeI       bslI     haeI                 scfI     aciI         mnlI             maeIII     bbvI      bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R

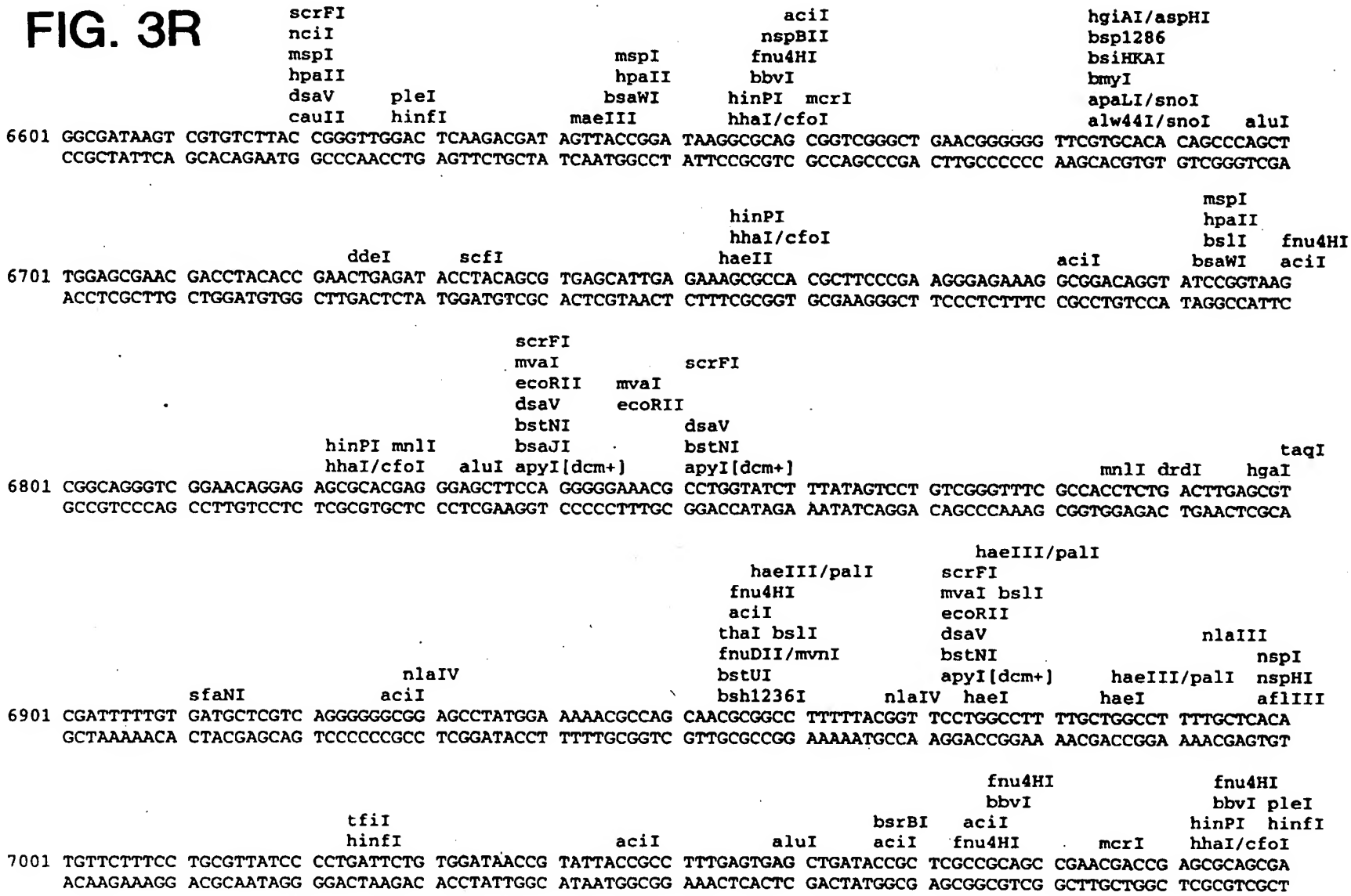
scrFI
nciI
mspI
hpaII
dsaV
cauII
pleI
hinfI
mspI
hpaII
bsaWI
maeIII
aciI
nspBII
fnu4HI
bbvI
hinPI mcrI
hhaI/cfoI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI
apaLI/snoI
alw44I/snoI
aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA
hinPI
hhaI/cfoI
mspI
hpaII
bslI
fnu4HI
ddeI
scfI
haeII
aciI
bsaWI
aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC
scrFI
mvaI
ecoRII
dsaV
bstNI
bsaJI
apyI[dcm+]
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
hinPI mnlI
hhaI/cfoI
aluI
taqI
mnlI drdI
hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA
haeIII/palI
haeIII/palI
fnu4HI
aciI
thaI bslI
fnuDII/mvnI
bstUI
bsh1236I
scrFI
mvaI bslI
ecoRII
dsaV
bstNI
apyI[dcm+]
nlaIV haeI
nlaIII
nspI
haeIII/palI
haeI
nspHI
aflIII
nlaIV
sfaNI
aciI
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT
fnu4HI
bbvI
fnu4HI
bbvI pleI
tfiI
hinfI
aciI
aluI
bsrBI
aciI
fnu4HI
mcrI
hinPI hinfI
hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT

FIG. 3S

```
                                                                      thaI
                                                                      fnuDII/mvnI
                                                                      bstUI
                                                                      bsh1236I
                                                                     hinPI
                                                                     hhaI/cfoI
                                                                    thaI
                                                                    fnuDII/mvnI
                    sapI  hinPI                                     bstUI   haeIII/palI       aluI
                    mboII hhaI/cfoI                                 bsh1236I          tru9I  pvuII
                    earI/ksp632I                         mnlI      bslI    eaeI  tfiI aseI/asnI/vspI
           mnlI  aciI    haeII                         aciI        aciI    cfrI  hinfI mseI   nspBII              bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                           scrFI
                                                                           mvaI
                                                                           ecoRII
                                                                           dsaV
                                          tru9I                      nlaIV bstNI
                             hinPI        mseI      maeIII           hgiCI apyI[dcm+]          mspI
         aciI                hhaI/cfoI   aseI/asnI/vspI  mnlI        banI bsaJI                hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                                        tru9I
                                                                        mseI
                                                                       aseI/asnI/vspI
            aciI                                                       xmnI
          bsrBI                                   aluI      nlaIII     asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT
```

>length: 7360

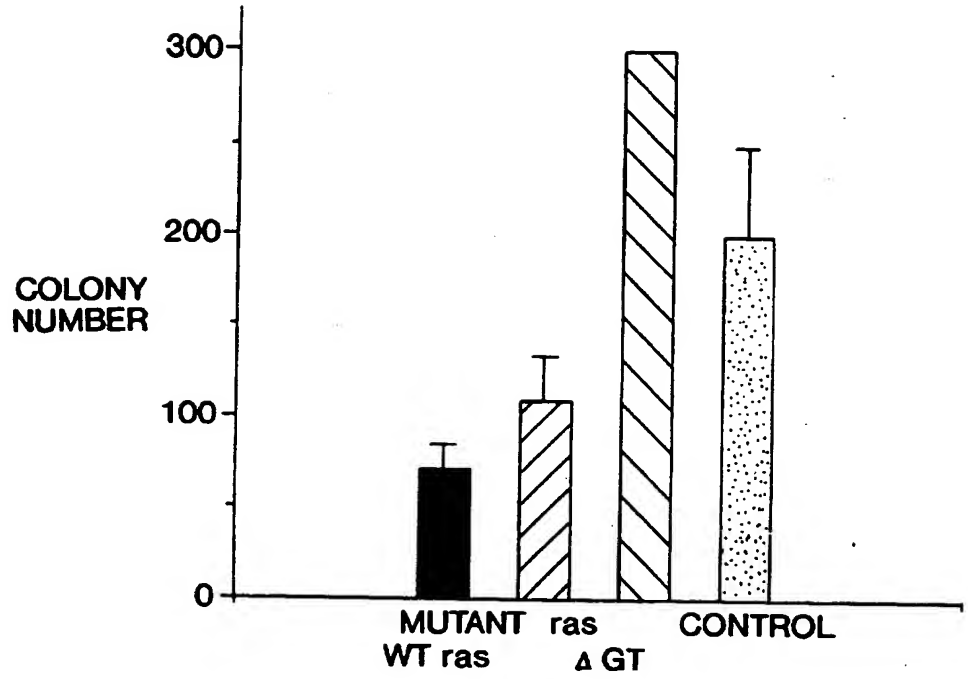
300
200
100
0
COLONY NUMBER
MUTANT ras
WT ras
Δ GT
CONTROL

FIG. 4

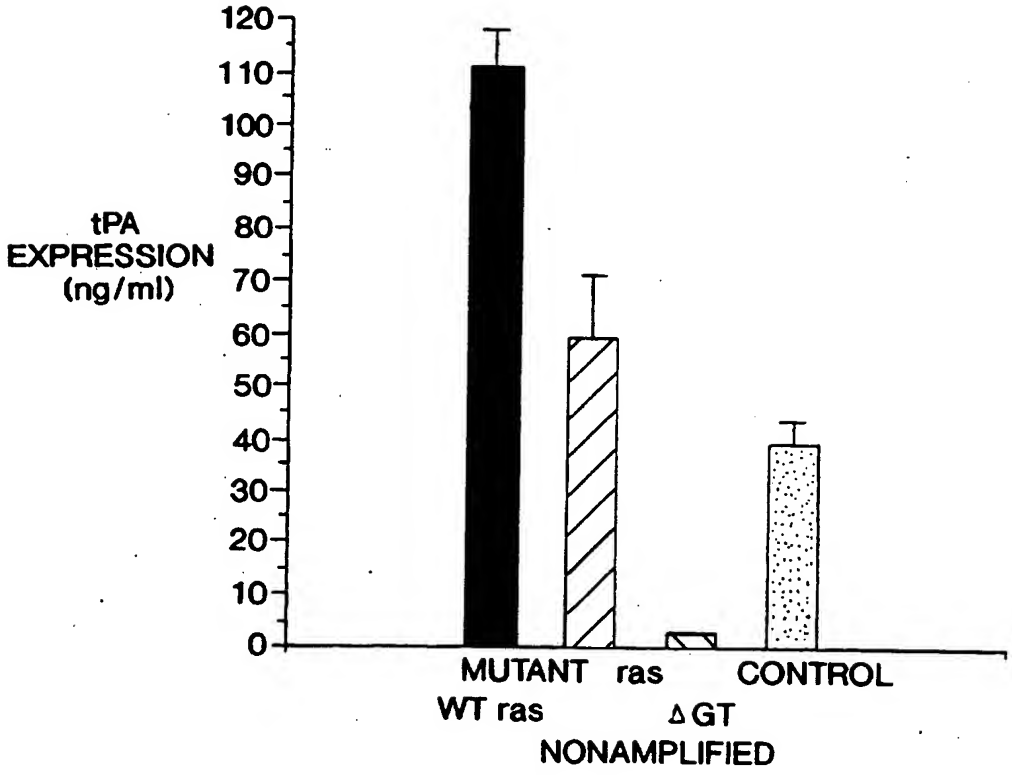
120
110
100
90
80
70
60
50
40
30
20
10
0
tPA EXPRESSION (ng/ml)
MUTANT ras
WT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5A

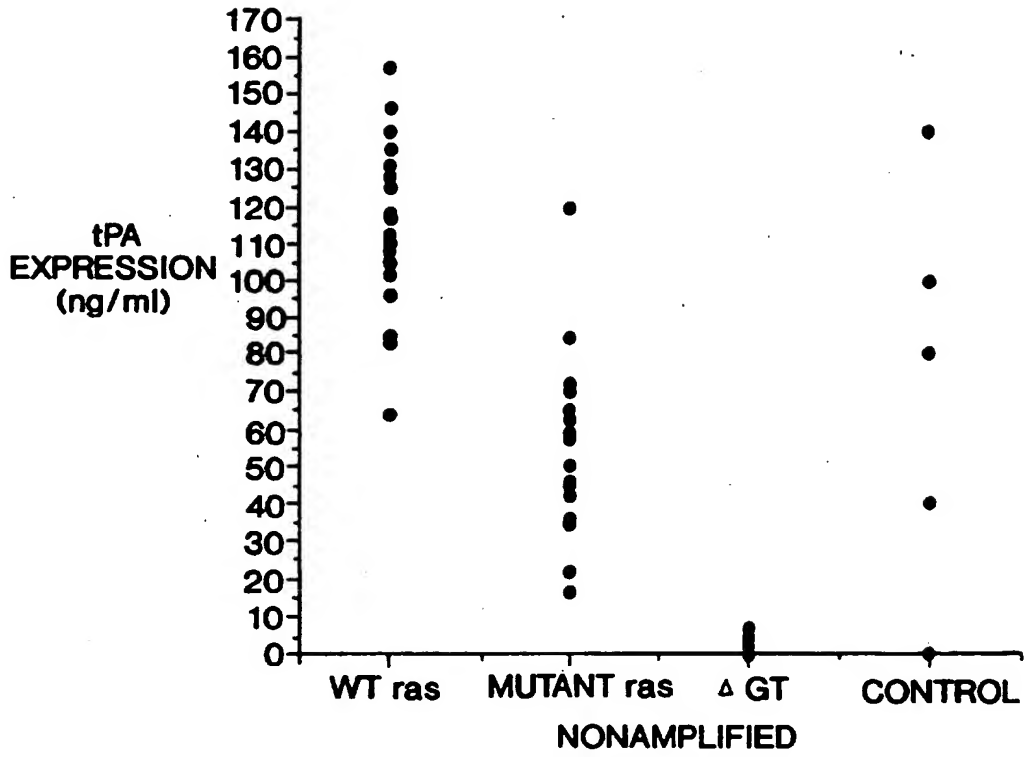
tPA
EXPRESSION
(ng/ml)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

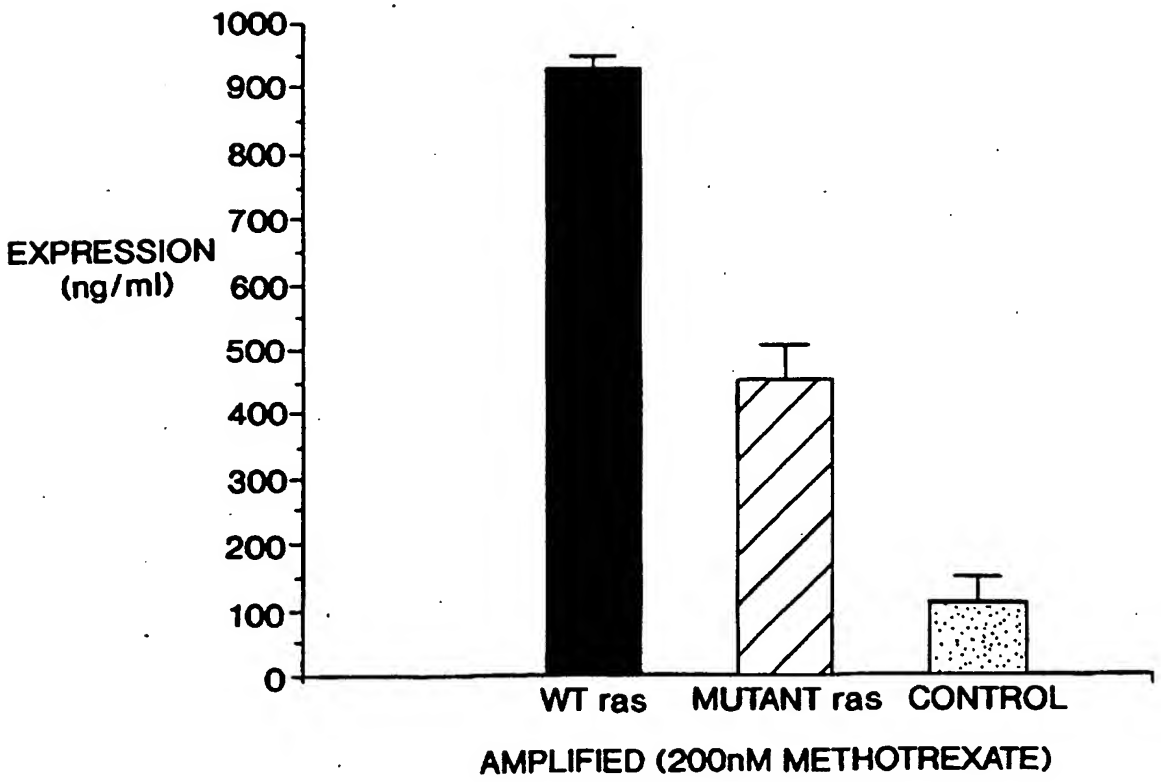
EXPRESSION
(ng/ml)
1000
900
800
700
600
500
400
300
200
100
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 6A

```
       aluI
      sstI
      sacI
      hgiJII
      hgiAI/aspHI
      ecl136II
      bsp1286
      bsiHKAI
      bmyI                           rmaI    tru9I                                                       thaI
      banII                          maeI    mseI                                                        fnuDII/mvnI
     taqI                           speI    aseI/asnI/vspI                                  bslI        bstUI
                                                                                                         bsh1236I
                                                                                                        aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                  scrFI
                  mvaI
                  ecoRII
                  dsaV
                aciI
               bglI bstNI                               maeII
              sau96I                                  hinlI/acyI
              haeIII/palI     aciI                    ahaII/bsaHI
              asuI   apyI[dcm+]           aciI        aatII        maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                         maeII
     hinlI/acyI                                                                                    hinlI/acyI
     ahaII/bsaHI                                    rsaI                     rsaI                  ahaII/bsaHI
     aatII                              bglI        csp6I        ndeI        csp6I                 aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

              scrFI
              mvaI
              ecoRII
          aciI                                                                                          nlaIII
        bglI  dsaV                                                                                styI
       sau96I bstNI          rsaI                                             maeII                    ncoI
       haeIII/palI           csp6I                                rsaI       snaBI                     dsaI hphI  aciI
       asuI   apyI[dcm+]   bsrI  nlaIII                           csp6I      bsaAI                     bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 6B

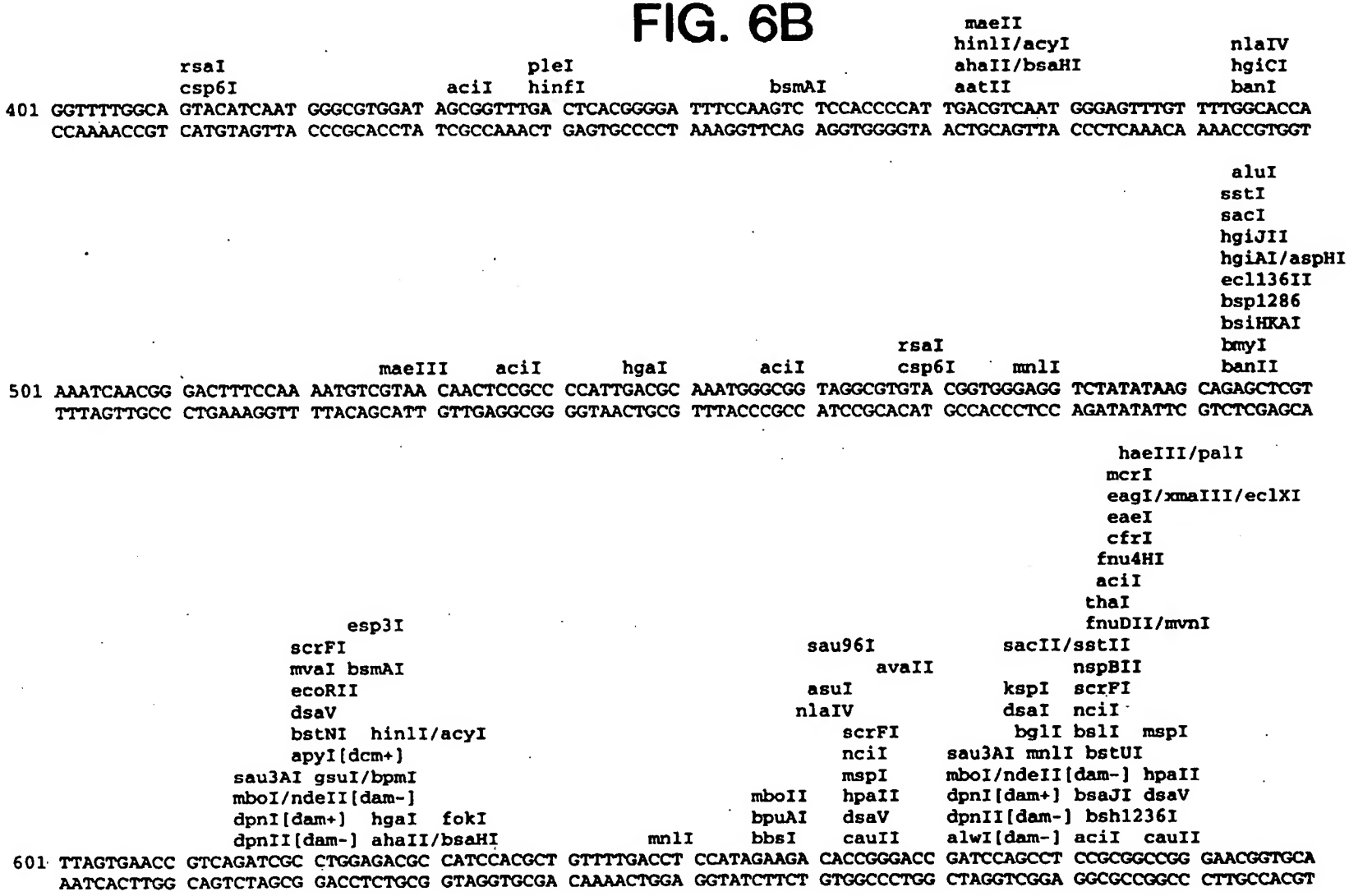
maeII
hinlI/acyI
nlaIV
rsaI
pleI
ahaII/bsaHI
hgiCI
csp6I
aciI
hinfI
bsmAI
aatII
banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT
aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
rsaI
bmyI
maeIII
aciI
hgaI
aciI
csp6I
mnlI
banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
fnu4HI
aciI
thaI
fnuDII/mvnI
esp3I
scrFI
sau96I
sacII/sstII
mvaI bsmAI
avaII
nspBII
ecoRII
asuI
kspI scrFI
dsaV
nlaIV
dsaI nciI
bstNI hinlI/acyI
scrFI
bglI bslI mspI
apyI[dcm+]
nciI
sau3AI mnlI bstUI
sau3AI gsuI/bpmI
mspI
mboI/ndeII[dam-] hpaII
mboI/ndeII[dam-]
mboII
hpaII
dpnI[dam+] bsaJI dsaV
dpnI[dam+] hgaI fokI
bpuAI
dsaV
dpnII[dam-] bsh1236I
dpnII[dam-] ahaII/bsaHI
mnlI
bbsI
cauII
alwI[dam-] aciI cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT

FIG. 6C

```
                 tfiI
             aciI
            thaI hinfI
            fnuDII/mvnI
            bstUI                                      aciI                                    fnu4HI
            bsh1236I                                  rsaI                                     bbvI
                                                      csp6I   scfI                              nspBII       taqI
                                                                        mnlI                   aciI       nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                       thaI
                                                                                       fnuDII/mvnI
                                                                                       bstUI
                                                                                       bsh1236I
                                                                                     mluI
                                                                         bsrBI       aflIII     rsaI
                 pflMI                              bsmAI                aciI      xmnI         csp6I
     sfaNI       bslI                               bsaI              mnlI   ddeI asp700       scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                                 scrFI
                                                                 mvaI
                                                                 ecoRII
       eco57I                                                    dsaV
      mboII                tfiI                                  bstNI                tfiI      tru9I        tru9I
      earI/ksp632I         hinfI                                 apyI[dcm+]        mboII  taqI  mseI         mseI
     mnlI                alwNI      hphI                       sexAI          ddeI    hinfI   ahaIII/draI   aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                                 aluI
                                                sstI
                                                sacI
                                                hgiJII
                                                hgiAI/aspHI
                                                ecl136II
                                                bsp1286
                                                bsiHKAI
                                                bmyI                                   tru9I            mspI
                                                banII                        sfaNI     mseI             hpaII
        ddeI                          bslI     mnlI               bstXI      fokI    aflII/bfrI        bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6D

```
                                                                                  haeIII/palI
                                                                                 haeI
                                                          scrFI          scrFI                     nlaIII
                                                          mvaI                 mvaI                         sau3AI
                                                          ecoRII         ecoRII                   mboI/ndeII[dam-]
                                                          dsaV   tfiI    dsaV    pleI             dpnI[dam+]
                                                          bstNI     nlaIII     bstNI    ddeI               dpnII[dam-]
          accI  nlaIII                 mnlI               apyI[dcm+]  hinfI    apyI[dcm+]  hinfI   maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                             mnlI
                                                                                        hinlI/acyI        scrFI
                                                                                        ahaII/bsaHI       mvaI
                                                                                     scrFI         ecoRII
                                                                                     mvaI    ecoNI        dsaV
                                                                                     ecoRII      sau96I
                                                                                     dsaV        avaII
                     maeII                                                           bstNI   bslI     asuI   mnlI
                    aflIII                                                           apyI[dcm+]     mnlI  bstNI
        apoI      maeIII                                        mnlI               bsaJI hgaI     ddeI   apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                  ppu10I
                                                              sfaNI                               nsiI/avaIII
        sfaNI                accI     mboII              mboII                        mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                          fnu4HI
       nlaIII                                             aciI
      styI                                               thaI
      ncoI                                               fnuDII/mvnI tru9I
      dsaI                           styI                bstUI      mseI                                    maeIII
      bsaJI                          bsaJI               bsh1236I  aseI/asnI/vspI                          hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6E

```
                                                                                                                        haeIII/palI
                                                                                                                      eaeI
                                               scrFI                                                            cfrI
                                               mvaI                                                                  mspI
                                               ecoRII                                                         hpaII
                                               dsaV                                                          scrFI
                                               bstNI                                                                nciI
                                               apyI[dcm+]          mnlI                               ecoRI  dsaV
                                             gsuI/bpmI             bsaJI                  taqI        apoI   cauII
     scfI        fokI                    maeIII      hincII/hindII                   claI/bsp106   bsaJI       aluI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                gsuI/bpmI
                                               scrFI
                                               mvaI
                                               ecoRII                                                          mnlI
                                               dsaV                                                            ecoNI
         mnlI                          nspBII  bstNI                                      ddeI                   sau96I
       haeIII/palI                  fnu4HI   hgiAI/aspHI                                 mnlI                    nlaIV
      sau96I                      fnu4HI aciI bsp1286                                   eco81I                   avaII
      asuI                        bbvI  fnu4HI bsiHKAI    aluI                          bsu36I/mstII/sauI        asuI  hphI
   nlaIII                     bspMI  bbvI      bmyI apyI[dcm+]                         bslI                  bsrI   bslI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

   rmaI
   maeI
  styI
  bsaJI
  blnI          mboII                 styI                                                  rsaI
  avrII         earI/ksp632I          bsaJI                  mnlI         taqI              csp6I          nlaIV
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6F

```
                                 scrFI
                                 nciI
                                 mspI
                                 hpaII
                                 dsaV
                                 cauII
                                xmaI/pspAI
                                smaI
                                scrFI
                                nciI
                                dsaV
                                cauII
                                bslI
                              sau96I
                              haeIII/palI
                              asuI
                           scrFI
                           mvaI bsaJI
                           ecoRII
                           dsaV                                   nlaIV
            rsaI           bstNI bsaJI              scfI              fnu4HI                        alwNI
            csp6I          bslI avaI                pstI              aciI         aciI          ddeI
           bsp1407I        apyI[dcm+]               bsgI           bsrBI       hphI   eco57I        mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                         scrFI
                                                       mboII             nciI
                                                      earI/ksp632I       mspI
          fnu4HI                                   sau3AI                hpaII
         aluI                                      mboI/ndeII[dam-]      dsaV                scfI               mspI
        pvuII                                      dpnI[dam+]            cauII               pstI               hpaII
        nspBII                                     dpnII[dam-]        sau96I                 bsgI            rsaI
      ddeI                                        bstYI/xhoII         avaII                 fnu4HI           csp6I
     mnlI bbvI                                    bglII               asuI     draIII       bbvI   mboII  bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6G

```
                                                                          hgiAI/aspHI                           hgiAI/aspHI
                                                                          bsp1286        scrFI            bsp1286
                                                                          bsiHKAI        mvaI                   bsiHKAI
                                                     mnlI           sau96I               ecoRII           bmyI
                                                  alwNI             avaII bmyI mnlI      dsaV             apaLI/snoI
                                                 fnu4HI             asuI  apaLI/snoI     bstNI                  alw44I/snoI
                                 bsrI      munI  bbvI       mnlI   nlaIV  alw44I/snoI    apyI[dcm+]       draIII
2001 CGGCATTATT GGAGTGAAAA CCTTTTCCAG TGCTTCAATT GCAGCCTCTG CCTCAATGGG ACCGTGCACC TCTCCTGCCA GGAGAAACAG AACACCGTGT
     GCCGTAATAA CCTCACTTTT GGAAAAGGTC ACGAAGTTAA CGTCGGAGAC GGAGTTACCC TGGCACGTGG AGAGGACGGT CCTCTTTGTC TTGTGGCACA

                                                                                     hgiAI/aspHI
                                                                                     bsp1286
                                                                                     bsiHKAI
                                                                                     bmyI
                                                                               gsuI/bpmI
                                                                              scrFI
                                                                              mvaI   apaLI/snoI
                                                                              ecoRII
                                                                              dsaV
                 bspMI                                 scfI                   bstNI  alw44I/snoI
       bspMI nlaIII           ddeI                bsmAI      maeIII           apyI[dcm+]
2101 GCACCTGCCA TGCAGGTTTC TTTCTAAGAG AAAACGAGTG TGTCTCCTGT AGTAACTGTA AGAAAAGCCT GGAGTGCACG AAGTTGTGCC TACCCCAGAT
     CGTGGACGGT ACGTCCAAAG AAAGATTCTC TTTTGCTCAC ACAGAGGACA TCATTGACAT TCTTTTCGGA CCTCACGTGC TTCAACACGG ATGGGGTCTA

                                                            aluI
                                                          sstI
                                                          sacI
                                                          hgiJII
                                                          hgiAI/aspHI
                                                          ecl136II
                                   nlaIV                  bsp1286                                          bsp1286
                  bsp1286    pleI  hgiCI                  bsiHKAI                                         nlaIV
             tru9I       mnlI  ddeI                       bmyI                 maeIII           nlaIII    hgiCI
             mseI bmyI ddeI  hinfI banI                   banII              hphI             nspI  dsaI banI
                                                                         draIII               nspHI bsaJI bmyI
2201 TGAGAATGTT AAGGGCACTG AGGACTCAGG CACCACAGAC AAGAGAGTTG AGCTCAAAAC CCCACTTGGT GACACAACTC ACACATGCCC ACGGTGCCCA
     ACTCTTACAA TTCCCGTGAC TCCTGAGTCC GTGGTGTCTG TTCTCTCAAC TCGAGTTTTG GGGTGAACCA CTGTGTTGAG TGTGTACGGG TGCCACGGGT

                                             bsp1286
                                            nlaIV                                             bsp1286
     hgiJII                                 hgiCI    hgiJII                                  nlaIV    hgiJII
     bsp1286                            dsaI bmyI    bsp1286                            dsaI hgiCI    bsp1286
     bmyI                          bsp1286  banI     bmyI                               bsaJI bmyI    bmyI
     banII         maeIII  mnlI    bmyI bsaJI        banII        maeIII   mnlI    nlaIII    banI     banII
2301 GAGCCCAAAT CTTGTGACAC ACCTCCCCCG TGCCCACGGT GCCCAGAGCC CAAATCTTGT GACACACCTC CCCCATGCCC ACGGTGCCCA GAGCCCAAAT
     CTCGGGTTTA GAACACTGTG TGGAGGGGGC ACGGGTGCCA CGGGTCTCGG GTTTAGAACA CTGTGTGGAG GGGGTACGGG TGCCACGGGT CTCGGGTTTA
```

FIG. 6H

```
                                                                            eam1105I
                                                                          sau96I
                                                                 scrFI
                                    bsp1286                      mvaI     avaII
                                  nlaIV                          ecoRII
                                  hgiCI                          dsaV
                              dsaI bmyI                          bstNI    asuI       mboII mboII
                              bsaJI                              bsaJI mnlI        bpuAI earI/ksp632I      styI
     maeIII    mnlI    nlaIII    banI    alwNI                   apyI[dcm+]        bbsI mnlI              bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

         sau96I
         nlaIV
         avaII
         asuI             maeII
        mspI             pmlI
        hpaII            eco72I                                          sau96I
       scrFI       mnlI  bsaAI                               drdI        avaII                 maeII
       nciI      ddeI maeIII                              mboII     mnlI                    bsaAI
       dsaV     eco81I  bbrPI                             bpuAI   bsaJI   bsrI              rsaI
       cauII    bsu36I/mstII/sauI          maeII          bbsI   avaI   asuI                csp6I            mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

                 acil
                thaI
                fnuDII/mvnI
                bstUI
                bsh1236I
               sacII/sstII                                                                scrFI
               nspBII                                                                     mvaI bsrI
               kspI                                                                       ecoRII
               dsaI                                                                       dsaV
               bsaJI                                                                      bstNI
               aciI                                                   mnlI      econI     bstNI                  rsaI
              fnu4HI  mnlI                maeII                  hgaI   hphI    bslI      apyI[dcm+]            csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```

FIG. 6I

```
                 bsmAI                                                                               rsaI
                                                                                                     csp6I
                bsaI            mnlI           taqI                                avaI            bsp1407I     bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

           scrFI
           nciI
           mspI
           hpaII
           dsaV
           cauII
          xmaI/pspAI
          smaI
          scrFI                   scrFI         scrFI
          nciI                    mvaI                mvaI
          dsaV                    ecoRII        ecoRII
          cauII                   dsaV          dsaV                                            aciI
          bsaJI                   bstNI               bstNI                            dsaI             nspBII
       fokI                       apyI[dcm+]          apyI[dcm+]                       bslI            fnu4HI fnu4HI
     bslI avaI  mnlI             sexAI           bspMI                                 bsaJI           bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                       dsaI
        mspI                                   pleI                 mnlI           hphI                    fnu4HI
        hpaII                     mnlI  nlaIII hinfI     nlaIV   mboII   scfI    aluI  bsaJI        bspMI  bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                                scrFI
                                                                                                                nciI
                                                                                                                mspI
                                                                                                                hpaII
                                                                                                                dsaV
                                                                                         scrFI                  cauII
                                                                                         nciI                   bsaJI
                                                                                         mspI                 haeIII/palI
                                    nlaIII                                               hpaII               mcrI
                                 ppu10I                             sapI                 dsaV                eagI/xmaIII/eclXI
         xmnI mboII              nsiI/avaIII                        mboII mnlI       bsmAI                   eaeI
         asp700      nlaIII     sfaNI   mnlI           aciI         earI/ksp632I  bslI   cauII               cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6J

```
         rmaI
      mnlI
   sau3AI
   mboI/ndeII[dam-]                            styI
   dpnI[dam+]                               aciI
   dpnII[dam-]  taqI                       fnu4HI   sau96I
   alwI[dam-]  salI    scfI               sfiI ncoI haeIII/palI
  nlaIV  maeI  hincII/hindII              haeIII/palI
  bstYI/xhoII  accI    pstI              eaeI  dsaI asuI                 aluI
  bamHI xbaI pleI      bsgI    aluI cfrI  bsaJI                       fnu4HI
  alwI[dam-] hinfI bspMI     hindIII bglI nlaIII                  bbvI          maeIII            sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                          sau3AI
                                                                                          mboI/ndeII[dam-]
                                                                                          dpnI[dam+]
                                                                                          dpnII[dam-]
                                                                                         pvuI/bspCI
                                                                                         mcrI
                                                                                       taqI[dam-]    tru9I
                                                                                      claI/bsp106[dam-]
                                                                                     sau3AI          mseI
                                                                                     mboI/ndeII[dam-]
                                                                                     dpnI[dam+] xmnI          hinPI
                                              rmaI                                    dpnII[dam-]  aseI/asnI/vspI
                                        bsmI  maeI                            nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                        rsaI
                                                        csp6I
         haeI                                          nlaIV
       styI                                            kpnI
       ncoI                                            hgiCI
       dsaI haeIII/palI                                banI                   aluI
  fnu4HI nlaIII                                        asp718     mnlI        pvuII
  bbvI  bsaJI              mnlI        mnlI            acc65I   ddeI aciI     nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6K

```
                 nlaIV                                                                          nlaIV
             scrFI                                                  scrFI        scrFI
             mvaI                                 ppu10I                  mvaI               mvaI
             ecoRII                   nsiI/avaIII             ecoRII       ecoRII
             dsaV                       nlaIII                   dsaV         dsaV
             bstNI                          sphI                       bstNI              bstNI
             apyI[dcm+]                     nspI sfaNI                 apyI[dcm+]         apyI[dcm+]
            bsaJI                           nspHI                     sexAI            bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

                     sfaNI
                   ppu10I
                   nsiI/avaIII
                  nlaIII
                 sphI
                 nspI                                                      aciI                               bslI
                 nspHI                        aciI     aciI  fokI            aciI bsrI aciI        aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC

                                                                                                             rmaI
                                                                                                            styI
                                                                                                            bsaJI
                                                   fnu4HI                                                   blnI
        nlaIII                                    sfiI mnlI                                                 avrII
       styI                                       haeIII/palI                                             haeIII/palI
       ncoI                                 bsaJI bglI          ddeI                                     stuI
       dsaI                                haeIII/palI bsaJI mnlI aluI                    mnlI           haeI
       bsaJI                            mnlI mnlI aciI     haeIII/palI                 mnlI             mnlI maeI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                             scrFI
                                                                             mvaI
                                                                             ecoRII
                       tru9I            haeIII/palI                          dsaV
                      hpaI             eaeI                                  bstNI
                      hincII/hindII    cfrI                  bsrI            apyI[dcm+]        tru9I       fnu4HI
                  aluI mseI aluI      bsrI              maeII maeIII        bsaJI   maeIII     mseI        bbvI
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```

FIG. 6L

```
                                                                                                              hinPI
                                                                                                              hhaI/cfoI
                                                                                                             nlaIV
                                                     sau3AI                                                  narI
                                         sau96I      mboI/ndeII[dam-]                                        kasI
                                         haeIII/palI                                                         hinlI/acyI
                                         asuI        dpnI[dam+]                                              hgiCI
                    aluI               mnlI          dpnII[dam-]                                             haeII      aciI
                   pvuII             mboII    aciI  pvuI/bspCI                                               banI     sfaNI
     fokI          nspBII            earI/ksp632I   mcrI                               bglI                  ahaII/bsaHI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                                               aciI
                                                                                                            fnu4HI
                                                                             hinPI                          aciI
                                                                            thaI                           thaI
                                                                            fnuDII/mvnI                    fnuDII/mvnI
                                                                            bstUI scfI     hinPI           bstUI
                                                                            bsh1236I       hhaI/cfoI hinPI
                                                                        rsaI hhaI/cfoI  fnu4HI   tru9I bsh1236I
                     sfaNI    aciI         aciI   maeII                 csp6I    bslI   aciI     mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

         fnu4HI
       hinPI
       hhaI/cfoI                            hinPI
      thaI                                  hhaI/cfoI
      fnuDII/mvnI                         rmaI                                                                 mspI
      bstUI                           hinPI  haeII                                                             hpaII
      bsh1236I    aciI                hhaI/cfoI    bsrBI                                                      naeI
     maeIII bbvI  maeIII             haeII maeI    aciI           mboII                         maeII   cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                    nlaIV
                   hgiJII
                   bsp1286                                    nlaIV                                        maeII    haeIII/palI
                   bmyI                                       hgiCI   taqI                                 draIII   sau96I
      aluI         banII          nlaIV                       banI mnlI                     hphI           bsaAI    asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6M

```
                                       maeII pleI           tru9I    pleI                                 bslI
                                      drdI   hinfI  maeII   mseI     hinfI            bsrI               bslI  avaI
4201 ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG
     TAGCGGGACT ATCTGCCAAA AAGCGGGAAA CTGCAACCTC AGGTGCAAGA AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT GGGATAGAGC

                                                                                              thaI
                                                                                              fnuDII/mvnI
                                                         tru9I                           tru9I   apoI tru9I       tru9I
                                                         mseI            tru9I           mseI bstUI   mseI        mseI
                                               haeIII/palI aluI         mseI    apoI     bsh1236I        sspI
4301 GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT AAAAAATGAG CTGATTTAAC AAAAATTTAA CGCGAATTTT AACAAAATAT
     CCGATAAGAA AACTAAATAT TCCCTAAAAC GGCTAAAGCC GGATAACCAA TTTTTTACTC GACTAAATTG TTTTTAAATT GCGCTTAAAA TTGTTTTATA

                         hgiAI/aspHI
                         bsp1286
                         bsiHKAI                                                                        hinPI
                         bmyI    ddeI                    aciI                                    maeIII         fnu4HI
       maeII             apaLI/snoI   rsaI              fnu4HI    tru9I                     maeII bsrI    nlaIII hhaI/cfoI
      psp1406I           alw44I/snoI  csp6I          sfaNI        mseI        aciI        bsaAI tth111I/aspI bbvI
4401 TAACGTTTAC AATTTTATGG TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAACTCCG CTATCGCTAC GTGACTGGGT CATGGCTGCG
     ATTGCAAATG TTAAAATACC ACGTGAGAGT CATGTTAGAC GAGACTACGG CGTATCAATT CGGTTGAGGC GATAGCGATG CACTGACCCA GTACCGACGC

                                                                                                  scrFI
                                  hinPI                         sfaNI                             nciI
                                  hhaI/cfoI                  mspI                                 mspI
                                 thaI                        hpaII                                hpaII      nspI
                                 fnuDII/mvnI                scrFI                                 dsaV       nspHI
                                 bstUI                      nciI                              esp3I       fnu4HI
                         nspBII  bsh1236I                   dsaV fokI                   maeIII bsmAI      bbvI
              aciI       aciI  hgaI       drdI              cauII   aciI            aluI    bslI  cauII   aluI nlaIII
4501 CCCCGACACC CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT
     GGGGCTGTGG GCGGTTGTGG GCGACTGCGC GGGACTGCCC GAACAGACGA GGGCCGTAGG CGAATGTCTG TTCGACACTG GCAGAGGCCC TCGACGTACA

                                       thaI
                                       fnuDII/mvnI
                                       bstUI
                                       bsh1236I
                                      hinPI
                                      hhaI/cfoI                         mnlI
                                    thaI                             haeIII/palI
                                    fnuDII/mvnI          mboII      sau96I                                   nlaIII
                                    bstUI mnlI           bpuAI      asuI                             tru9I  rcaI
         mnlI    hphI      hphI     bsh1236I             bbsI      eco0109I/draII                    mseI   bspHI
4601 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GTATTCTTGA AGACGAAAGG GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT
     CAGTCTCCAA AAGTGGCAGT AGTGGCTTTG CGCGCTCCGT CATAAGAACT TCTGCTTTCC CGGAGCACTA TGCGGATAAA AATATCCAAT TACAGTACTA
```

FIG. 6N

```
                                                          nlaIV
                                                        aciI
                                                       thaI
                                                       fnuDII/mvnI
                     hinlI/acyI                        bstUI                                                    rcaI
                     ahaII/bsaHI                       bsh1236I                                                 bspHI
                     aatII                            hinPI                                                 bsrBI
                 ddeI maeII                           hhaI/cfoI                                             aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

                                                     mboII                                                 fnu4HI
       bsmAI                           sspI          earI/ksp632I                                          aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                             hgiAI/aspHI
                                                                             bsp1286
                                                                  sau3AI     bsiHKAI                     sau3AI
                                                                  mboI/ndeII[dam-]                       mboI/ndeII[dam-]
                                                                  dpnI[dam+] bmyI                        dpnI[dam+]
                                                                  dpnII[dam-]                           bstYI/xhoII
                                                             eco57I          apaLI/snoI              bsrI dpnII[dam-]
                         hphI             hphI          sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI   alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                           aciI
              sau3AI                                                                      thaI
              mboI/ndeII[dam-]              maeII                                         fnuDII/mvnI
              dpnI[dam+]                   psp1406I          hgiAI/aspHI                  bstUI
              dpnII[dam-]                 xmnI               bsp1286 tru9I                bsh1236I            hinlI/acyI
       aciI   alwI[dam-]                  asp700             bsiHKAI mseI                hinPI                hgaI
     nspBII  bstYI/xhoII                mboII                bmyI   ahaIII/draI          hhaI/cfoI            ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

      scrFI
      nciI
      mspI
      hpaII                                                       rsaI
      dsaV                    aciI                                csp6I   bsrI
      cauII      bcgI  mcrI  fnu4HI          ddeI                scaI  hphI  maeIII      sfaNI     fokI   nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 6O

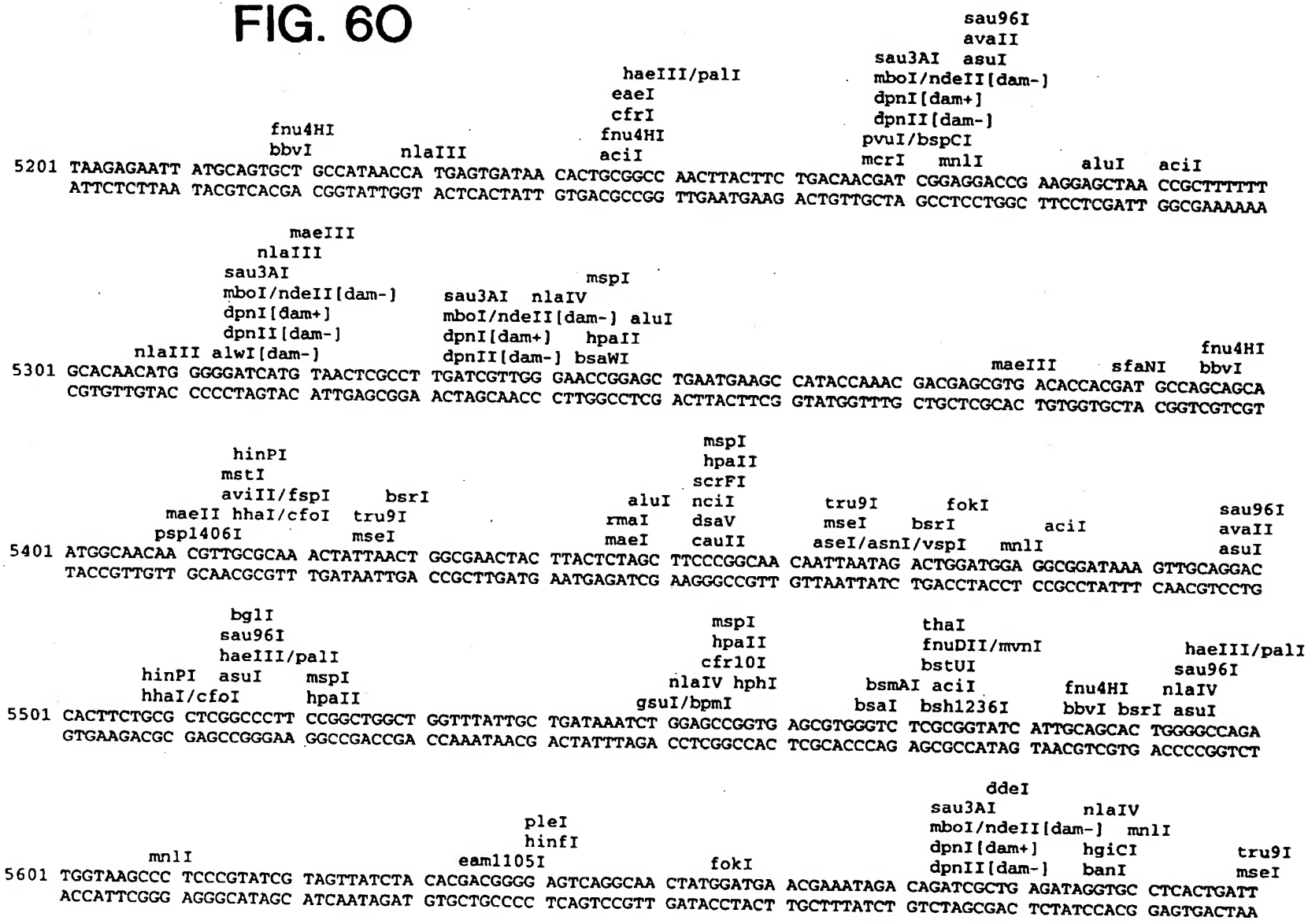

FIG. 6P

```
                                                                                     rmaI    sau3AI
                                                                                 sau3AI hphI mboI/ndeII(dam-)
                                                                                 mboI/ndeII(dam-)
                                                                                 dpnI(dam+)  dpnI(dam+)
                                                                                 dpnII(dam-) dpnII(dam-)
                                                       tru9I            ahaIII/draI maeI     alwI(dam-)
                                                       mseI         tru9I tru9I bstYI/xhoII bstYI/xhoII
           maeIII                                     ahaIII/draI   mseI  mseI  alwI(dam-) mboII(dam-)
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                              sau3AI
                                                                              mboI/ndeII(dam-)
                                                                              dpnI(dam+)  sau3AI
                                                                              dpnII(dam-) mboI/ndeII(dam-)
                                                                             bstYI/xhoII  dpnI(dam+)
                                                                      sau3AI alwI(dam-)   dpnII(dam-)
          nlaIII           maeII                                      mboI/ndeII(dam-)    alwI(dam-)
         rcaI           tru9I                  hgaI                   dpnI(dam+) mboII(dam-)
         bspHI          mseI                ddeI                      dpnII(dam-)        bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                             sau3AI
      thaI                                                   mboI/ndeII(dam-)
      fnuDII/mvnI                                            dpnI(dam+)
      bstUI                                                  dpnII(dam-)
      bsh1236I                                              alwI(dam-)
     hinPI     fnu4HI                          aciI         mspI                                bsrI
     hhaI/cfoI bbvI                   aciI   nspBII         hpaII     aluI                    maeIII  eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

             hinPI                   rmaI             haeIII/palI
             hhaI/cfoI               maeI     bslI   haeI                scfI   aciI      mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 6Q

```
                                                         scrFI                                      aciI
                                                         nciI                                     nspBII
                           fnu4HI                        mspI                         mspI        fnu4HI
                  alwNI    bbvI                          hpaII                        hpaII       bbvI
                 bsrI  fnu4HI                            dsaV     pleI               bsaWI      hinPI mcrI
            maeIII     bbvI    bsrI                      cauII    hinfI         maeIII          hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

                  hgiAI/aspHI
                  bsp1286
                  bsiHKAI
                  bmyI                                                                          hinPI
                  apaLI/snoI                                                                    hhaI/cfoI
                  alw44I/snoI   aluI                          ddeI      scfI                   haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                             scrFI
                                                                             mvaI          scrFI
                                                                             ecoRII  mvaI
                            mspI                                             dsaV    ecoRII
                            hpaII                                            bstNI         dsaV
                            bslI  fnu4HI                    hinPI mnlI       bsaJI         bstNI
              aciI         bsaWI  aciI                      hhaI/cfoI   aluI apyI[dcm+]    apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                                  haeIII/palI
                                                                                                fnu4HI
                                                                                                aciI
                                                                                               thaI bslI
                                                                                               fnuDII/mvnI
                                   taqI                            nlaIV                       bstUI
                   mnlI drdI       hgaI           sfaNI          aciI                          bsh1236I      nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6R

```
                haeIII/palI
               haeI
              scrFI
              mvaI bslI
              ecoRII                nlaIII
              dsaV                  nspI
              bstNI          haeIII/palI  nspHI                    tfiI                                                    bsrBI
              apyI[dcm+]     haeI         aflIII                   hinfI                    aciI          aluI     aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                                  thaI
                                                                                                  fnuDII/mvnI
                                                                                                  bstUI
                                                                                                  bsh1236I
                                                                                                 hinPI
                                                                                                 hhaI/cfoI
                                                                                                thaI
                                                                                                fnuDII/mvnI
        fnu4HI              fnu4HI                        sapI  hinPI                           bstUI   haeIII/palI
        bbvI                bbvI pleI                     mboII hhaI/cfoI                       bsh1236I           tru9I
       aciI                 hinPI  hinfI                  earI/ksp632I              mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
      fnu4HI       mcrI     hhaI/cfoI          mnlI   aciI    haeII          aciI       aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                                  scrFI
                                                                                                  mvaI
                                                                                                  ecoRII
                                                                                                  dsaV
         aluI                                                  tru9I                            nlaIV bstNI
         pvuII                                          hinPI       mseI      maeIII             hgiCI apyI[dcm+]
         nspBII                      bsrI     aciI      hhaI/cfoI  aseI/asnI/vspI  mnlI        banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                                  tru9I
                                                                                                  mseI
                                                                                                 aseI/asnI/vspI
                mspI                          aciI                                              xmnI
                hpaII                        bsrBI                          aluI      nlaIII    asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT
```

>length: 6889

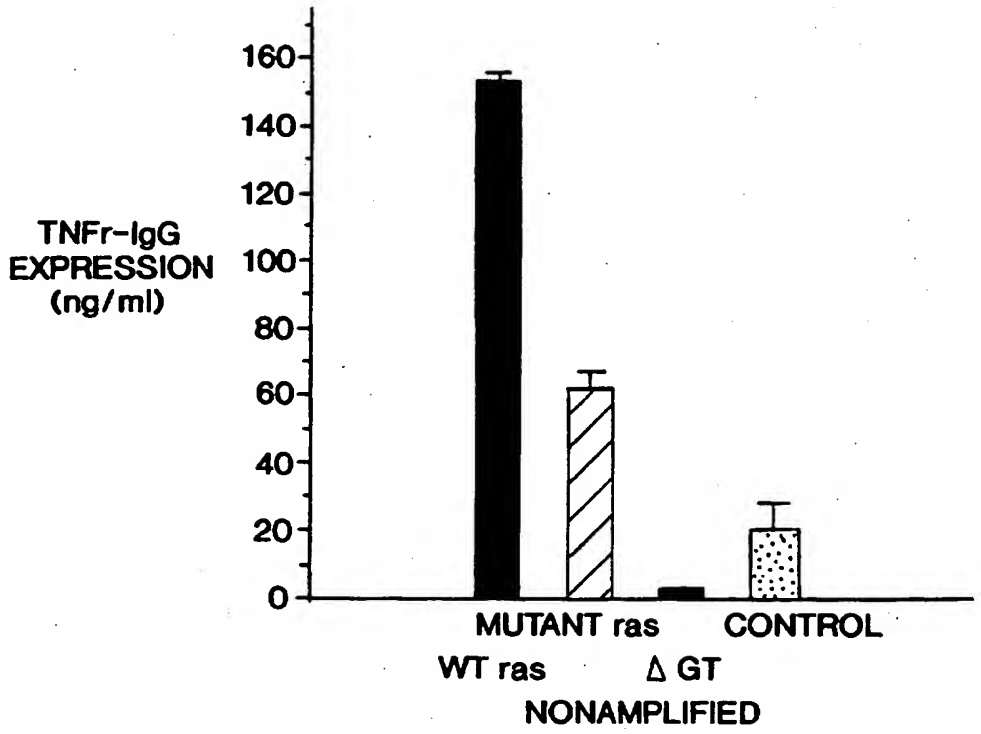
TNFr-IgG
EXPRESSION
(ng/ml)
160
140
120
100
80
60
40
20
0
MUTANT ras
CONTROL
WT ras
Δ GT
NONAMPLIFIED

FIG. 7A

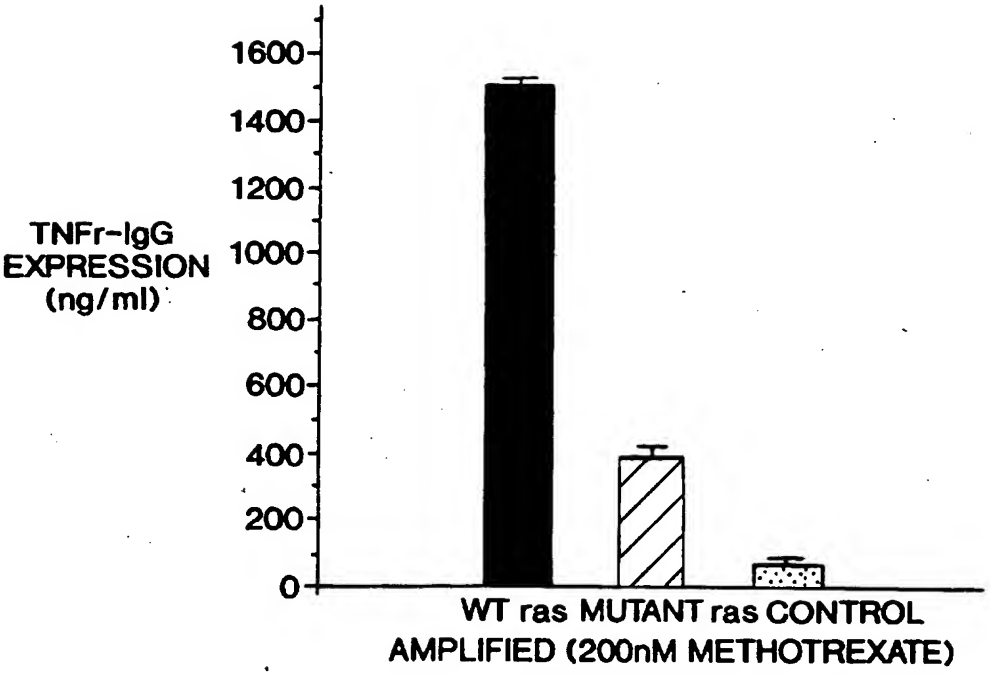
TNFr-IgG
EXPRESSION
(ng/ml)
1600
1400
1200
1000
800
600
400
200
0
WT ras MUTANT ras CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 7B

FIG. 8

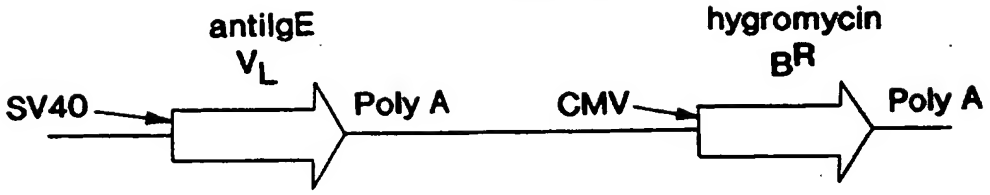
antiIgE
V_L
hygromycin
B^R
SV40
Poly A
CMV
Poly A

FIG. 11

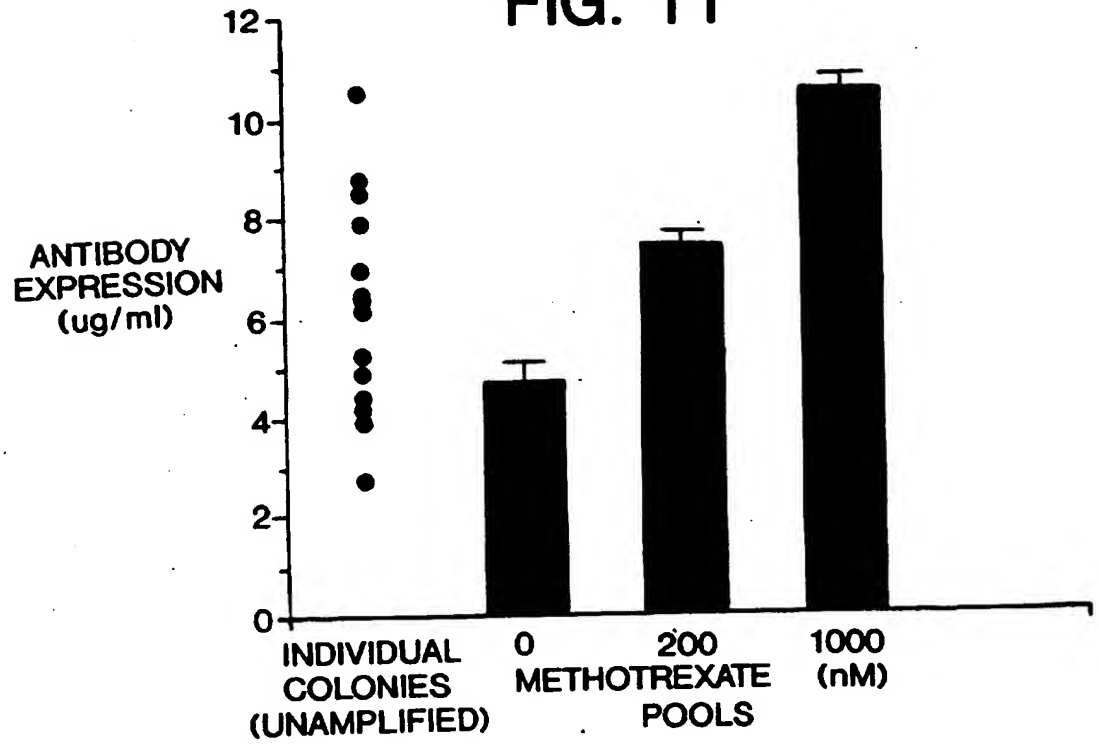
ANTIBODY EXPRESSION (ug/ml)
12
10
8
6
4
2
0
INDIVIDUAL COLONIES (UNAMPLIFIED)
0
200
1000
(nM)
METHOTREXATE POOLS

FIG. 9A

```
     aluI
    sstI
    sacI                                       aluI
    hgiJII                               sau3AI pvuII                                              nlaIV
    hgiAI/aspHI                          mboI/ndeII(dam-)                                      scrFI
    ecl136II                             dpnI(dam+)                                            mvaI
    bsp1286                             pvuI/bspCI                                             ecoRII
    bsiHKAI                          pleI dpnII(dam-)                                          dsaV
    bmyI                             hinfI  taqI(dam-)                                         bstNI
    banII                         rmaI   mcrI    nspBII                                        apyI(dcm+)
   taqI                           maeI  taqI(dam-)                                            bsaJI
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                              nlaIV
            sfaNI                scrFI      scrFI                                sfaNI
           ppu10I                mvaI           mvaI                                    ppu10I
           nsiI/avaIII           ecoRII     ecoRII                              nsiI/avaIII
          nlaIII                 dsaV       dsaV                               nlaIII
         sphI                    bstNI          bstNI                                  sphI
         nspI                    apyI(dcm+)     apyI(dcm+)                             nspI
         nspHI                  sexAI           bsaJI                                  nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                   nlaIII
                                                                                  styI
                                                                                  ncoI
                                  aciI                                         bslI dsaI
           aciI         aciI  fokI                 aciI bsrI  aciI             aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                              rmaI
                                                                             styI
           fnu4HI                                                            bsaJI                         haeIII/palI
           bglI                                                              blnI                        mcrI
          sfiI                                                               avrII               aluI    eagI/xmaIII/eclXI
          haeIII/palI                                                      haeIII/palI          rmaI     eaeI
        mnlI   mnlI       ddeI                                            stuI                  maeI     cfrI
    haeIII/palI bsaJI  mnlI   aluI                       mnlI             haeI                 nheI     mspI
  mnlI bsaJI aciI   haeIII/palI                       mnlI              mnlI maeI             aluI     hpaII
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 9B

```
                              tfiI
    scrFI                     hinfI
    nciI                   aciI
    mspI                  thaI                                                                    fnu4HI
    hpaII                 fnuDII/mvnI                         aciI                                bbvI
    dsaV                  bstUI                     maeII  rsaI                                 nspBII
    cauII                 bsh1236I               maeIII    csp6I  scfI            mnlI          aciI       nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                              haeIII/palI
                                                                             haeI
                                                                           scrFI
                                                                           mvaI     bsrBI
                                                                           ecoRII
                                                                           dsaV
                                                                           bstNI    aciI                  rsaI
                                     pflMI                  bsmAI     apyI[dcm+]        xmnI         csp6I
    taqI           sfaNI             bslI                   bsaI     bsaJI  mnlI  ddeI  asp700      scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                        eco57I                                            dsaV
                       mboII                 tfiI                         bstNI                   tfiI      tru9I
                      earI/ksp632I           hinfI                        apyI[dcm+]              hinfI     mseI
                     mnlI                  alwNI   hphI                  sexAI           ddeI mboII   taqI ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                            sstI
                                                            sacI
                                                            hgiJII
                                                            hgiAI/aspHI
                                                            ecl136II
                                                            bsp1286
                                                            bsiHKAI
             tru9I                                          bmyI                                  tru9I
             mseI         ddeI                              banII                                aflII/bfrI
            aseI/asnI/vspI                       bslI   mnlI aluI          bstXI      fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9C

```
                                                                                               haeIII/palI
                                                                                              haeI
                                                                       scrFI         scrFI
                                                                       mvaI                mvaI
                                                                       ecoRII        ecoRII
         mspI                                                          dsaV   tfiI   dsaV
         hpaII                                                         bstNI    nlaIII     bstNI     ddeI pleI
        bsaWI                accI nlaIII             mnlI              apyI[dcm+]   hinfI  apyI[dcm+]     hinfI
 801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
     TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                                     hgaI
                                                                                                    hinlI/acyI
                                                                                                    ahaII/bsaHI
                                                                                                 scrFI
                 nlaIII                                                                          mvaI     mnlI
             sau3AI                                                                              ecoRII
             mboI/ndeII[dam-]                                                                    dsaV
             dpnI[dam+]                  maeII                                                   bstNI    ecoNI
             dpnII[dam-]               aflIII                                                    apyI[dcm+]    mnlI
     maeIII alwI[dam-]  apoI       maeIII                                       mnlI           bsaJI     bslI ddeI
 901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
     CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

         scrFI
         mvaI
         ecoRII
         dsaV
         bstNI
         apyI[dcm+]
      sau96I
      avaII                                                           sfaNI
      asuI   mnlI       sfaNI                 accI     mboII             mboII                       mnlI    aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                     styI
                                                     bsaJI
                                               sau3AI
                       nlaIII                  mboI/ndeII[dam-]
                      styI                     dpnI[dam+]
                      ncoI                     dpnII[dam-]                aluI     tru9I
     ppu10I           dsaI                     alwI[dam-]               fnu4HI     mseI
     nsiI/avaIII      bsaJI                   bstYI/xhoII               bbvI      aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```

FIG. 9D

```
                                                                                        sau96I
                                                                                        avaII
                                                                                        asuI
                                                                                     scrFI
                                                                                     mvaI
                                                                                     ecoRII
                                                                                     dsaV
                                                                                     bstNI                              ecoRI
           maeIII                                                                    apyI[dcm+]       mnlI        taqI   apoI
          hphI    scfI        fokI                                           bslI  bsaJI              bsaJI      claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                             scrFI
        nlaIII                                                                                               mvaI
       styI                                                                                                  ecoRII
       pflMI                                                                                                 dsaV
       ncoI                                                                                                  bstNI fnu4HI
       dsaI                                                          rsaI               aluI                 apyI[dcm+]
       bslI fokI                           rmaI                 gsuI/bpmI              pvuII      pleI      haeI      bbvI
       bsaJI        nlaIII   fokI          maeI               bsrI    csp6I            nspBII     hinfI   aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9E

```
                                                                                                                                  scrFI
                                                                                                                                  nciI
                                                                                                                                  mspI
                                                                                                                                  hpaII
                                                                                                                                  dsaV
                                                                                                                                  cauII
                                                                                                                                  bslI
                                                                                                                                 xmaI/pspAI
                                                                                                                                 smaI
                                                                                                                                 scrFI
                                                                                                                                 nciI
                                                                                                                                 dsaV
                                                                                                                                 cauII           scrFI
                                                                                                                              sau96I             mvaI
                                                                                                                  sau3AI      nlaIV              ecoRII
                                                                                 mspI                             mboI/ndeII[dam-]               dsaV
                                                                            hpaII                          dpnI[dam+]     bslI           bstNI
                                                                                   mroI                           dpnII[dam-]   bsaJI            bslI
                                                                           bspMII                       nlaIV         haeIII/palI        apyI[dcm+]
                                                                           bspEI                        bstYI/xhoII     avaI          sau96I
                                                                                 bsaWI                        bamHI     eco0109I/draII haeIII/palI
                                                                               mnlI                           alwI[dam-] asuI                asuI
                                                                           hphI  accIII       aluI      bsrI alwI[dam-]  bsaJI            eco0109I/draII
     hgiJII
     bsp1286
     bmyI
   scrFI
   mvaI banII
   ecoRII
   dsaV
   bstNI
   bsaJI
   apyI[dcm+]                             alwNI
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                                       sau3AI
                                       mboI/ndeII[dam-]
                                       dpnI[dam+]                                                     thaI
                         maeII         dpnII[dam-]                                                    fnuDII/mvnI
                   taqI snaBI         alwI[dam-]              hgaI       haeIII/palI                  bstUI  tfiI
                  claI/bsp106       mspI                     xmaI       sau96I                        bsh1236I
              sfaNI      bsaAI      hpaII taqI[dam-]         maeI       asuI                        nruI     hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9F

```
                                                                                   scrFI
                                                                                   mvaI
                                                                                   ecoRII
                                                                                   dsaV
                                                                                   bstNI
                                                                                   apyI[dcm+]
                                                                                hinPI
                                                                                hhaI/cfoI
                                                    mnlI                       nlaIV
                                                 xhoI                          narI
                                                 paeR7I                        kasI
                                                 avaI                          hinlI/acyI
     scfI                                    hgiAI/aspHI                       hgiCI
     pstI                                    bsp1286                           haeII
     bsgI                    mnlI            bsiHKAI  fnu4HI                   banI
   bspMI                   ddeI drdI         bmyI taqI bbvI                    ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                  sau96I
                                  haeIII/palI
                                 sau96I
                                 nlaIV
                                 hgiJII                      nlaIV
                                 bsp1286                     hgiCI                                          scrFI
        scrFI                  bsp120I                    banI                                              mvaI
        mvaI                     bmyI                       scrFI                                           ecoRII
        ecoRII                 banII                    mvaI                                                dsaV
        dsaV                   asuI                     ecoRII                                          bstNI
        bstNI hphI                apaI               mboII   dsaV          hgiAI/aspHI                  bsaJI
        apyI[dcm+] bsmAI    haeIII/palI eco0109I/draII       bstNI              bsp1286                sau96I
       bsaJI maeIII      mnlI         styI asuI     bpuAI   apyI[dcm+]  mnlI    bsiHKAI            haeIII/palI
    nlaIV   bstEII esp3I bsaJI mnlI   bsaJI         bbsI   bsaJI     mnlI       bmyI mnlI              asuI
                                                                                                        fnu4HI
                                                                                         bsp1286 aciI apyI[dcm+]
                                                                                         bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```

FIG. 9G

```
          scrFI                                            hinPI
          mvaI                                                  nlaIV
          ecoRII                                          narI
          ecoNI                                                 kasI                hgiAI/aspHI
          dsaV                hphI                    hinlI/acyI          bsp1286      mspI
          bstNI                   mspI                      hgiCI               bsiHKAI      hpaII
          bslI                    hpaII                     haeII               bmyI        scrFI
          apyI[dcm+]            cfr10I                      banI          fnu4HI            nciI
      fnu4HI                    bsaWI  tth111I/aspI         ahaII/bsaHI   aciI  apaLI/snoI  dsaV
      bbvI            bslI  ageI maeIII             ddeI hhaI/cfoI  nspBII  alw44I/snoI cauII      scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                               nlaIV
      ddeI pleI                                       fnu4HI   hgiCI
     mnlI  hinfI                               mnlI   bbvI     banI                        tfiI
     eco81I           mnlI  fnu4HI  maeIII bsp1286 rmaI       bsp1286                      hinfI                styI
     bsu36I/mstII/sauI ddeI bbvI   hphI    bmyI    maeI aluI  bmyI                    maeII                     bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                                eam1105I
                                                                                               sau96I
                                                                                       scrFI
                                                                                       mvaI    avaII
                                                                                       ecoRII
                         hgiJII                                                        dsaV
                         bsp1286                        nlaIII                         bstNI   asuI       mboII mboII
                         bmyI                          nspI        bsp1286             bsaJI  nlaIV      bpuAI earI/ksp632I
                         banII          maeIII         nspHI       bmyI  alwNI         apyI[dcm+]        bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9H

```
                                             sau96I
                                             nlaIV
                                             avaII
                                           mspI
                                          scrFI
                                          nciI
                                    sau3AI hpaII
                                    mboI/ndeII[dam-]
                                    dpnI[dam+]                         nlaIII
                                 nlaIII    dsaV            mnlI   nspI
                                rcaI       cauII         ddeI maeIII
               styI             bspHI[dam-]  asuI       eco81I     nspHI
               bsaJI          mnlI   dpnII[dam-]        bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC

                    drdI  mnlI
               mboII   ddeI
               bpuAI   eco81I
     maeII     bbsI    bsu36I/mstII/sauI
     GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                               aciI
                                               thaI
                                               fnuDII/mvnI
                                               bstUI
                                               bsh1236I
                                              sacII/sstII
                                              nspBII
                                              kspI
          maeII                               dsaI
        rsaI                                  bsaJI
        csp6I                                 aciI
     bsrI bsaAI          mnlI                fnu4HI  mnlI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA

                       rsaI
                       csp6I
     rsaI          maeII                    mnlI      econI
     csp6I         bsaAI           hgaI     hphI      bslI
     ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

       scrFI
       mvaI bsrI
       ecoRII
       dsaV
       bstNI                     rsaI         bsmAI
       apyI[dcm+]                csp6I       bsaI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG

                                                 fnu4HI
       mnlI        taqI                          bbvI
     AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9I

```
                                                scrFI
                                                ncII
                                                mspI
                                                hpaII
                                                dsaV
                                                cauII
                                               xmaI/pspAI
                                               smaI
                                               scrFI                                      scrFI
                                               ncII                      mvaI                         scrFI
                                               dsaV                      ecoRII                  mvaI
                                               cauII                     dsaV                    ecoRII
                      rsaI                fokI    mboII                  bstNI                        dsaV
                      csp6I          bslI bsaJI                          apyI[dcm+]                   bstNI
        avaI         bsp1407I        bslI avaI earI/ksp632I              sexAI                   bspMI apyI[dcm+]
2401 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
     GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

          dsaI                          mspI
          bslI                      fnu4HI                                          pleI                 mnlI
          bsaJI                     bbvI hpaII                        mnlI          hinfI     nlaIV  mboII  scfI    aluI
2501 ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
     TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                                 bpuAI
                                                 bbsI                        nlaIII
        dsaI                                    maeII                     ppu10I                              sapI
        bsaJI                       fnu4HI   xmnI mboII                   nsiI/avaIII                         mboII  mnlI
      hphI              bspMI       bbvI     asp700         nlaIII       sfaNI    mnlI                        earI/ksp632I bslI
2601 GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
     CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                                           styI
         scrFI                                    taqI                   aciI
         ncII                                    salI                   fnu4HI   sau96I
         mspI                          rmaI      hincII/hindII         sfiI ncoI haeIII/palI
         hpaII                         maeI      accI     scfI         haeIII/palI
         dsaV                       sau96I  pleI      pstI           eaeI  dsaI asuI                  aluI
         cauII                      haeIII/palI       bsgI    aluI cfrI   bsaJI                    fnu4HI
      bsmAI                         asuI    hinfI bspMI     hindIII bglI nlaIII                    bbvI          maeIII
2701 CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
     GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9J

```
                                                                                                       taqI[dam-]
                                                                                                      claI/bsp106[dam-]
                                                                                                     sau3AI
                                                                                                     mboI/ndeII[dam-]
                                                                                                     dpnI[dam+]
                                                          rmaI                                       dpnII[dam-]
        sfaNI    apoI                               bsmI  maeI                             nlaIII    alwI[dam-]
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

          tru9I                                                        rsaI
       xmnI                                                            csp6I
      sau3AI  aseI/asnI/vspI     styI                                 nlaIV
      mboI/ndeII[dam-]           ncoI                                 kpnI
      dpnI[dam+]             fnu4HI   haeI                            hgiCI
      dpnII[dam-]            bbvI  dsaI haeIII/palI                   banI                           aluI
     pvuI/bspCI mseI      hinPI    bsaJI                              asp718    mnlI              pvuII
     mcrI    asp700       hhaI/cfoI nlaIII          mnlI      mnlI    acc65I  ddeI aciI           nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                               nlaIV
                            scrFI                                                         scrFI
                            mvaI                              ppu10I                         mvaI
                            ecoRII                        nsiI/avaIII                     ecoRII
                            dsaV                          nlaIII                          dsaV
                            bstNI                            sphI                            bstNI
                            apyI[dcm+]                       nspI sfaNI                      apyI[dcm+]
                           bsaJI                             nspHI                         sexAI         bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

        nlaIV                           sfaNI
     scrFI                      ppu10I
     mvaI                            nsiI/avaIII
     ecoRII                    nlaIII
     dsaV                     sphI
     bstNI                         nspI                                                     aciI
     apyI[dcm+]                    nspHI                     aciI         aciI  fokI         aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9K

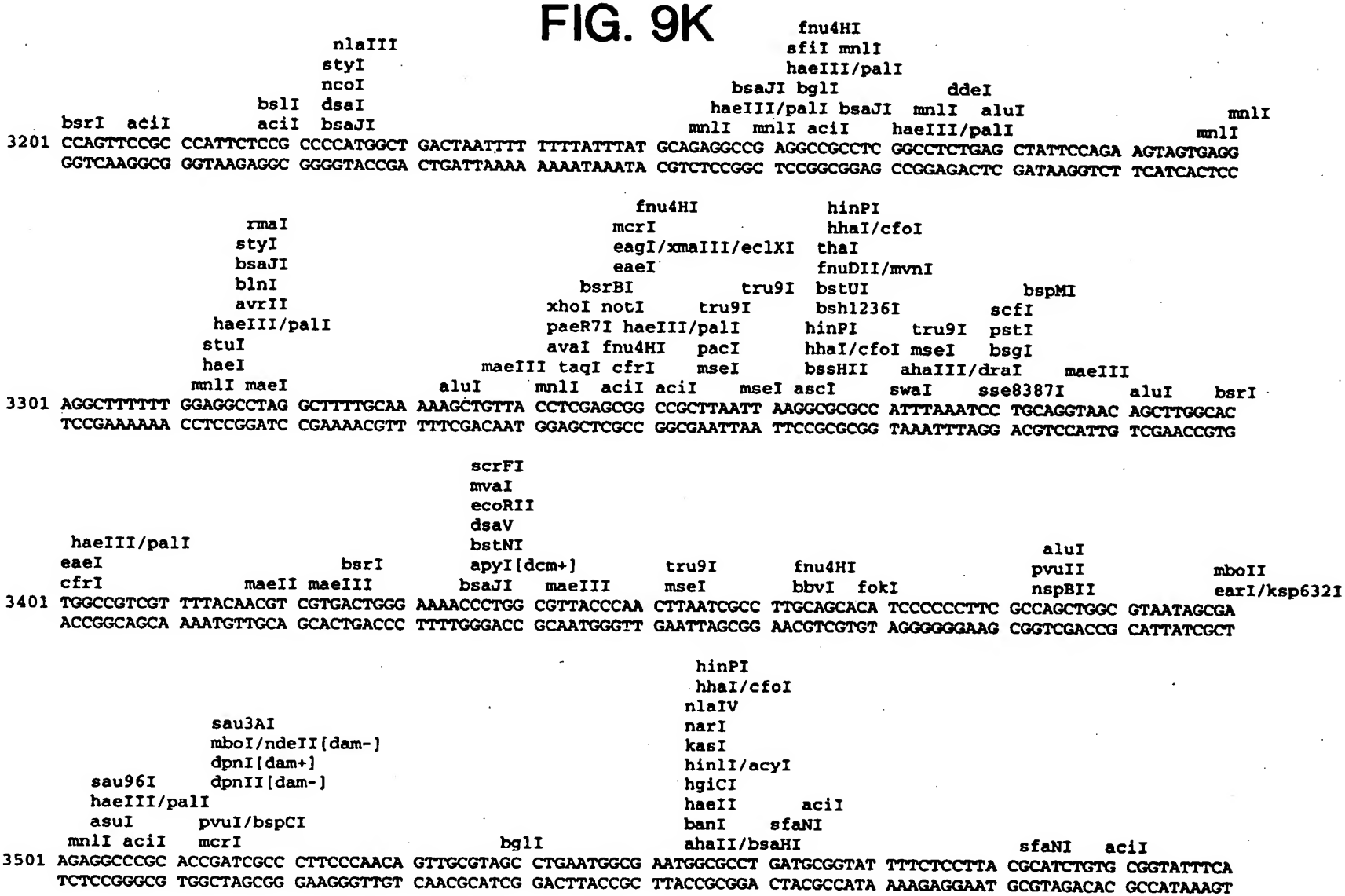

FIG. 9L

```
                                                                  aciI
                                    hinPI                      fnu4HI                  fnu4HI
                                    hhaI/cfoI                  aciI                  hinPI
                                   thaI                       thaI                   hhaI/cfoI
                                   fnuDII/mvnI                fnuDII/mvnI           thaI
                                   bstUI          hinPI       bstUI                 fnuDII/mvnI
                                   bsh1236I       hhaI/cfoI hinPI                   bstUI                   hinPI
                                rsaI      scfI fnu4HI   tru9I bsh1236I              bsh1236I     aciI           hhaI/cfoI
       aciI  maeII              csp6I   bslI   aciI     mseI hhaI/cfoI         maeIII  bbvI  maeIII             haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

            hinPI                                                                              nlaIV
            hhaI/cfoI                                          mspI                            hgiJII
           haeII                                               hpaII                           bsp1286
         rmaI    bsrBI                                        naeI                             bmyI
         maeI    aciI           mboII                maeII  cfr10I            aluI           banII          nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

                      nlaIV                                       maeII   haeIII/palI
                      hgiCI  taqI                                draIII   sau96I                                maeII
                      banI mnlI                   hphI        bsaAI    asuI                                  drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

          pleI          tru9I     pleI                              bslI
          hinfI  maeII  mseI      hinfI           bsrI             bslI   avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                         thaI                                       hgiAI/aspHI
                                                         fnuDII/mvnI                                bsp1286
                                                         bstUI               maeII             bsiHKAI
                      tru9I                          tru9I               tru9I                 bmyI    ddeI
                      mseI           tru9I       mseI    apoI tru9I       mseI                 apaLI/snoI  rsaI
           haeIII/palI     aluI    mseI    apoI    bsh1236I mseI    sspI  psp1406I            alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9M

```
                                                                                                                    hinPI
                                                                                                                    hhaI/cfoI
                                                                                                                   thaI
                                                                                      hinPI                        fnuDII/mvnI
       aciI                                          maeIII                      fnu4HI                            bstUI
      fnu4HI       tru9I                           maeII bsrI      nlaIII   hhaI/cfoI             nspBII         bsh1236I
    sfaNI          mseI          aciI            bsaAI tth111I/aspI bbvI              aciI        aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                                       thaI
                                                                                                                       fnuDII/mvnI
                                                                                                                       bstUI
                                                         scrFI                                                         bsh1236I
          mspI                                           nciI                                                        hinPI
          hpaII                                          mspI                                                        hhaI/cfoI
         scrFI                                      bsmAI            nspI                                           thaI
         nciI     aciI                             esp3I hpaII fnu4HI                                               fnuDII/mvnI
         dsaV sfaNI                               bslI   dsaV aluI nspHI                                            bstUI
 drdI    cauII fokI               aluI maeIII            cauII bbvI nlaIII   mnlI    hphI     hphI                  bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                     mnlI
                   haeIII/palI                                                              maeII
       mboII      sau96I                                             nlaIII                 hinlI/acyI
       bpuAI      asuI                                      tru9I   rcaI                    ahaII/bsaHI
 mnlI  bbsI      eco0109I/draII                             mseI    bspHI             ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

        nlaIV
       aciI
      thaI
      fnuDII/mvnI
      bstUI
      bsh1236I                                                       rcaI
     hinPI                                                           bspHI
     hhaI/cfoI                                                     bsrBI    bsmAI
                                                                   aciI nlaIII                                  sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

      mboII                                                        fnu4HI
      earI/ksp632I                                                 aciI                      hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9N

```
                                            hgiAI/aspHI
                                            bsp1286
                                  sau3AI    bsiHKAI                                sau3AI  nspBII        sau3AI
                                  mboI/ndeII[dam-]                                 mboI/ndeII[dam-]      mboI/ndeII[dam-]
                                  dpnI[dam+] bmyI                                  dpnI[dam+]            dpnI[dam+]
                                  dpnII[dam-]                                     bstYI/xhoII            dpnII[dam-]
                        eco57I              apaLI/snoI                        bsrI dpnII[dam-]           alwI[dam-]
      hphI         sfaNI  mboII[dam-]       alw44I/snoI maeIII taqI         alwI[dam-]   aciI  bstYI/xhoII           mboII
4601 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
     GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                                   scrFI
                                                                                   nciI
                                                        aciI                       mspI
                                                        thaI                       hpaII
                                                        fnuDII/mvnI                dsaV
        maeII              hgiAI/aspHI                  bstUI                      cauII
       psp1406I            bsp1286   tru9I              bsh1236I               hinlI/acyI
      xmnI                 bsiHKAI   mseI               hinPI                  hgaI                          aciI
      asp700               bmyI    ahaIII/draI          hhaI/cfoI              ahaII/bsaHI    bcgI   mcrI   fnu4HI
4701 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
     TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                              rsaI
                              csp6I   bsrI                                                       fnu4HI
        ddeI                  scaI hphI   maeIII     sfaNI     fokI    nlaIII                    bbvI       nlaIII
4801 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
     GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                                 sau96I
                                                 avaII                                              maeIII
                                          sau3AI asuI                                             nlaIII
                haeIII/palI               mboI/ndeII[dam-]                                       sau3AI
               eaeI                       dpnI[dam+]                                             mboI/ndeII[dam-]   sau3AI
               cfrI                       dpnII[dam-]                                            dpnI[dam+]         mboI/ndeII[dam-]
              fnu4HI                     pvuI/bspCI                                              dpnII[dam-]        dpnI[dam+]
              aciI                       mcrI    mnlI         aluI   aciI              nlaIII alwI[dam-]            dpnII[dam-]
4901 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
     TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 90

```
                                                                                                      hinPI
          mspI                                                                                       mstI
          hpaII                                                                                      aviII/fspI     bsrI
          bsaWI                                                                    fnu4HI      maeII hhaI/cfoI   tru9I
     nlaIV    aluI                                     maeIII      sfaNI       bbvI       psp1406I              mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                    mspI
                    hpaII                                                                            bglI
                   scrFI                                                                             sau96I
          aluI     nciI       tru9I      fokI                          sau96I                       haeIII/palI
        rmaI       dsaV       mseI   bsrI          aciI                avaII           hinPI   asuI     mspI
        maeI       cauII      aseI/asnI/vspI   mnlI                    asuI            hhaI/cfoI        hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                     mspI              thaI
                     hpaII             fnuDII/mvnI              haeIII/palI
                    cfr10I             bstUI                   sau96I
                nlaIV  hphI        bsmAI aciI          fnu4HI nlaIV
              gsuI/bpmI           bsaI bsh1236I        bbvI bsrI asuI            mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                                   ddeI
                                              sau3AI        nlaIV
       pleI                                   mboI/ndeII[dam-]  mnlI
       hinfI                                  dpnI[dam+]    hgiCI          tru9I
  eam1105I              fokI                  dpnII[dam-]   banI           mseI       maeIII
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                   rmaI     sau3AI
                                                sau3AI hphI mboI/ndeII[dam-]
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]  dpnI[dam+]
                                         tru9I  dpnII[dam-] dpnII[dam-]
                   tru9I               ahaIII/draI maeI     alwI[dam-]           nlaIII               maeII
                   mseI             tru9I     bstYI/xhoII bstYI/xhoII            rcaI               tru9I
                 ahaIII/draI        mseI mseI alwI[dam-] mboII[dam-]             bspHI              mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9P

```
                                           sau3AI
                                           mboI/ndeII[dam-]
                                           dpnI[dam+]  sau3AI           thaI
                                           dpnII[dam-] mboI/ndeII[dam-]
                                          bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                  sau3AI  alwI[dam-]   dpnII[dam-]      bstUI
                                  mboI/ndeII[dam-]     alwI[dam-]       bsh1236I
                 hgaI             dpnI[dam+] mboII[dam-]               hinPI        fnu4HI
             ddeI                 dpnII[dam-]         bstYI/xhoII      hhaI/cfoI    bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                sau3AI
                                mboI/ndeII[dam-]
                                dpnI[dam+]
                                dpnII[dam-]
                               alwI[dam-]
             aciI              mspI                                   bsrI              hinPI
   aciI  nspBII                hpaII      aluI                    maeIII    eco57I      hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                              fnu4HI
                                                                                         alwNI   bbvI
  rmaI              haeIII/palI                                                        bsrI  fnu4HI
  maeI       bslI  haeI                scfI     aciI          mnlI                 maeIII    bbvI     bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                scrFI                                       aciI                       hgiAI/aspHI
                nciI                                      nspBII                       bsp1286
                mspI                      mspI           fnu4HI                        bsiHKAI
                hpaII                     hpaII          bbvI                          bmyI
                dsaV     pleI            bsaWI         hinPI mcrI                      apaLI/snoI
                cauII    hinfI        maeIII           hhaI/cfoI                        alw44I/snoI   aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 9Q

```
                                                                                                   mspI
                                                       hinPI                                       hpaII
                                                       hhaI/cfoI                                   bslI     fnu4HI
                      ddeI      scfI                  haeII                             aciI      bsaWI    aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                 scrFI
                                 mvaI         scrFI
                                 ecoRII  mvaI
                                 dsaV    ecoRII
                                 bstNI        dsaV
                  hinPI  mnlI    bsaJI        bstNI                                                      taqI
                  hhaI/cfoI  aluI apyI[dcm+]  apyI[dcm+]                               mnlI drdI   hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                haeIII/palI
                                                         haeIII/palI      scrFI
                                                        fnu4HI            mvaI
                                                        aciI              ecoRII
                                                        thaI bslI         dsaV                  nlaIII
                                                        fnuDII/mvnI       bstNI bslI                 nspI
                               nlaIV                    bstUI             apyI[dcm+]     haeIII/palI nspHI
            sfaNI            aciI                       bsh1236I     nlaIV haeI          haeI        aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                 fnu4HI             fnu4HI
                                                                                 bbvI               bbvI pleI
                      tfiI                                              bsrBI   aciI           hinPI    hinfI
                      hinfI                   aciI          aluI        aciI  fnu4HI     mcrI  hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9R

```
                                                          thaI
                                                          fnuDII/mvnI
                                                          bstUI
                                                          bsh1236I
                                                        hinPI
                                                        hhaI/cfoI
                                                       thaI
                                                       fnuDII/mvnI
                 sapI  hinPI                           bstUI             tru9I    aluI
                 mboII hhaI/cfoI                       bsh1236I haeIII/palI      pvuII
                 earI/ksp632I                 mnlI   bslI      eaeI  tfiI aseI/asnI/vspI
       mnlI   aciI     haeII              aciI       aciI      cfrI  hinfI mseI   nspBII                bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                scrFI
                                                                mvaI
                                                                ecoRII
                                                                dsaV
                             tru9I                          nlaIV bstNI
                  hinPI      mseI       maeIII              hgiCI apyI[dcm+]            mspI
     aciI         hhaI/cfoI aseI/asnI/vspI mnlI             banI bsaJI                  hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                             tru9I
                                                             mseI
                                                            aseI/asnI/vspI
        aciI                                                xmnI
      bsrBI                        aluI      nlaIII         asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT

>length: 6557
```

FIG. 10A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ecl136II
     bsp1286
     bsiHKAI
     bmyI                                                                                          thaI
     banII                                 rmaI      tru9I                                         fnuDII/mvnJ
    taqI                                   maeI      mseI                                          bstUI
                                          speI      aseI/asnI/vspI                                 bsh1236I
                                                                                       bslI       aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                 scrFI
                 mvaI
                 ecoRII
                 dsaV
              aciI
            bglI bstNI                            maeII
           sau96I                                hinlI/acyI
           haeIII/palI    aciI                   ahaII/bsaHI
           asuI  apyI[dcm+]              aciI    aatII        maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

     maeII                                                                                      maeII
    hinlI/acyI                                                                                 hinlI/acyI
    ahaII/bsaHI                                      rsaI                        rsaI          ahaII/bsaHI
    aatII                              bglI          csp6I           ndeI        csp6I         aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
       aciI                                                                                         nlaIII
     bglI  dsaV                                                                             styI
     sau96I bstNI         rsaI                                                maeII                ncoI
     haeIII/palI          csp6I                                     rsaI      snaBI                dsaI hphI  aciI
     asuI  apyI[dcm+]   bsrI  nlaIII                                csp6I     bsaAI                bsaJI    sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 10B

```
                                                                                   maeII
                                                                                  hinlI/acyI               nlaIV
               rsaI                          pleI                                 ahaII/bsaHI              hgiCI
               csp6I                  aciI   hinfI                 bsmAI          aatII                    banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                             rsaI                        bmyI
                                maeIII    aciI       hgaI        aciI        csp6I     mnlI              banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                haeIII/palI
                                                                                               mcrI
                                                                                               eagI/xmaIII/eclXI
                                                                                               eaeI
                                                                                               cfrI
                                                                                              fnu4HI
                                                                                              aciI
                                                                                             thaI
                             esp3I                                                           fnuDII/mvnI
                        scrFI                                            sau96I        sacII/sstII
                        mvaI bsmAI                                             avaII        nspBII
                        ecoRII                                           asuI          kspI scrFI
                        dsaV                                            nlaIV          dsaI nciI
                        bstNI hinlI/acyI                                    scrFI       bglI bslI mspI
                        apyI[dcm+]                                          nciI   sau3AI mnlI bstUI
                    sau3AI gsuI/bpmI                                        mspI   mboI/ndeII[dam-] hpaII
                    mboI/ndeII[dam-]                                mboII   hpaII  dpnI[dam+] bsaJI dsaV
                    dpnI[dam+] hgaI fokI                            bpuAI   dsaV   dpnII[dam-] bsh1236I
                    dpnII[dam-] ahaII/bsaHI                mnlI     bbsI    cauII  alwI[dam-] aciI cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
               tfiI                                                                             fnu4HI
           aciI                                                                                 aciI
          thaI hinfI                                            bstXI                           thaI
          fnuDII/mvnI                        aciI                sau96I    styI                 fnuDII/mvnI tru9I
          bstUI                  maeII rsaI         pleI scfI haeIII/palI                      bstUI      mseI
          bsh1236I        maeIII    csp6I   scfI hinfI     asuI      bsaJI               bsh1236I  aseI/asnI/vspI
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                     sau96I
                                                                                                     avaII
                                                                                                       asuI
                                                                                                  scrFI
                                                                                                  mvaI
                                                                                                  ecoRII
                                                                                                  dsaV
                                                                                                  bstNI
                                        maeIII                                                    apyI[dcm+]
                                       hphI   scfI         fokI                              bslI bsaJI
801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
    GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                      hinlI/acyI
                                                      ahaII/bsaHI
                                                      aatII
                                                   thaI                        taqI[dam-]
                                                   fnuDII/mvnI               sau3AI                bsmAI
                 aluI taqI                         bstUI                     mboI/ndeII[dam-]     esp3I
     mnlI       hindIII                          aciI maeII                  dpnI[dam+]      tth111I/aspI
     bsaJI   ddeI     claI/bsp106            hphI bsh1236I    taqI           dpnII[dam-] taqI   hgaI         sfaNI
901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
    TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                             hinPI
      aluI              tfiI                                                             fnu4HI
    fnu4HI              hinfI                 taqI                                       bbvI
    bbvI       mnlI  mboII                  aluI         mnlI                 aciI      aluI hhaI/cfoI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                         hinPI
                                         hhaI/cfoI    mspI
                                        thaI          hpaII
                                       aciI          mroI
                                    haeIII/palI      bspMII
                                   mcrI fnuDII/mvnI  bspEI
     sau3AI                        eagI/xmaIII/eclXI bsaWI
     mboI/ndeII(dam-)              eaeI bstUI     tfiI
     dpnI(dam+)                    cfrI bsh1236I  hinfI                      ecoRI
     dpnII(dam-)              sfaNI  fnu4HI  bslI   accIII                   apoI                       sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                           mcrI
                                                                                                           eagI/xmaIII/eclXI
                                                                                                           eaeI
                                                                                       styI               fnu4HI
        hgiAI/aspHI                                                           thaI     ncoI       sau3AI  aciI
        bsp1286                                                               fnuDII/mvnI         mboI/ndeII(dam-)
        bsiHKAI                                                     fnu4HI    bstUI    dsaI       dpnI(dam+)   sau3AI
        bmyI                                                        bbvI  mcrI       haeIII/palI  dpnII(dam-)  mboI/ndeII(dam-)
        apaLI/snoI                                                scfI   mspI bsh1236I bsaJI sfaNI    fnu4HI   dpnI(dam+)
        alw44I/snoI    maeII                                nspBII   pstI   hpaII    mnlI nlaIII   pvuI/bspCI haeIII/palI
      bslI draIII   maeIII        bspMI                     aciI     bsgI  cfr10I aciI haeI   fokI mcrI bbvI cfrI dpnII(dam-)
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                       sau96I                                            thaI       sau3AI
                                       avaII                                             fnuDII/mvnI       nlaIII
                                       asuI                                              bstUI      mboI/ndeII(dam-)
                             sau96I   rsrII/cspI                                         bsh1236I   dpnI(dam+)
                    aciI     haeIII/palI  aciI      tfiI                                hinPI       dpnII(dam-)
      ddeI        bsrBI      asuI     cpoI      hinfI             nlaIII        ndeI hhaI/cfoI   alwI(dam-)
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                                      hinPI                                                     draIII
                                                      hhaI/cfoI                                               nlaIV
                                                     thaI                          bsaJI                      hgiCI
                                                     fnuDII/mvnI                 haeIII/palI                  banI
                               tth111I/aspI          bstUI            sfaNI      sau96I                     mspI
         bsrI           drdI            hgaI     bsh1236I    taqI   aluI         asuI mnlI      bslI    hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

```
                 aciI
                thaI
                fnuDII/mvnI
          hgiAI/aspHI
          bsp1286
          bsiHKAI
          bmyI  bstUI
          apaLI/snoI                                        aciI
          alw44I/snoI                                      fnu4HI
                                                          haeIII/palI
                                                         eaeI        aciI        gsuI/bpmI            tfiI bslI
      mnlI      bsh1236I   nlaIV                         cfrI      nspBII      bsrI      mnlI         hinfI
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
     TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT

                                                                                                        fnu4HI
                                                                                                       thaI
                                                                                                       fnuDII/mvnI
                                                                                                       bstUI
                                                                                                       bsh1236I
                                                                                                      sacII/sstII
                                                                                                      nspBII
                                                                                                      kspI
                                                                                    mspI              dsaI
                                                                                    hpaII             bsaJI
                                                                                   mroI               aciI
                                                                                   bspMII            fnu4HI
                                                                                   bspEI            sau3AI aciI
                                             dsaI                                  bsaWI            mboI/ndeII[dam-]
                                          haeIII/palI                   rsaI     aciI  fokI         dpnI[dam+]
                                  mboII  mnlI bsaJI              fnu4HI    csp6I  bsrBI  sfaNI   aluI   dpnII[dam-]
       mnlI              mboII    gsuI/bpmI                      bbvI    maeII    taqI   mnlI  accIII   alwI[dam-]
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
     TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC

          scrFI
          nciI
          mspI
          hpaII                                                                       aluI
          dsaV                                                                       fnu4HI           hgaI
          cauII                                                                      bbvI      hinPI    taqI  drdI
      nlaIV                aciI                       aluI   hincII/hindII taqI  sfaNI      hhaI/cfoI  sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
     GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC
```

FIG. 10F

```
             nlaIV                                                  haeIII/palI
            mspI                                                   mcrI
            hpaII  scrFI                                           eagI/xmaIII/eclXI
            bslI   nciI                                            eaeI
           mroI    mspI                                            cfrI
           bspMII  hpaII                                          fnu4HI
           bspEI[dam-]                                            aciI
           bsaWI   dsaV                                          thaI
           accIII[dam-]                                          fnuDII/mvnI
          sau3AI   cauII                                         bstUI
          mboI/ndeII[dam-]                                       bsh1236I    sau96I                rsaI
          dpnI[dam+]                                             bsh1236I    sau96I                rsaI
          dpnII[dam-]                rsaI                       hinPI       avaII                 csp6I
          alwI[dam-]                 csp6I         aciI         hhaI/cfoI   asuI                  scaI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                              scrFI
                                                                              nciI
                                                                              mspI
                                                                              hpaII
                                                                              dsaV
                                                                             xmaI/pspAI
                                                                             smaI
                                                                             scrFI
                                                                             nciI
                                                                             dsaV
                                                                             cauII
                                                                             bsaJI
                                                                             avaI
                                                                            bsaJI
                                                                          sau3AI                        sau96I
                                                                          mboI/ndeII[dam-]       aciI   haeIII/palI
                                                                          dpnI[dam+]            fnu4HI  asuI
                                                                          dpnII[dam-]           bglI  nlaIII
                                                                          alwI[dam-]           sfiI  styI
                                                                         nlaIV cauII           eaeI  ncoI
          hinlI/acyI                                          mcrI       bstYI/xhoII           cfrI  dsaI
          hgaI                  mnlI                         bslI        bamHI bsaJI ecoRI   taqI haeIII/palI
          ahaII/bsaHI         bsaJI                        sfaNI         alwI[dam-]  apoI   claI/bsp106 bsaJI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
               aluI
             fnu4HI
             bbvI          maeIII                  sfaNI    apoI                                          rmaI
                                                                                                   bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                            sau3AI
                                            mboI/ndeII[dam-]
                                            dpnI[dam+]
                                            dpnII[dam-]
                                           pvuI/bspCI
                                           mcrI
                                          taqI[dam-]  tru9I                haeIII/palI
                                         claI/bsp106[dam-]                haeI
                                        sau3AI        mseI        fnu4HI styI
                                        mboI/ndeII[dam-]          bbvI   ncoI
                                        dpnI[dam+] xmnI          hinPI   dsaI
                                        dpnII[dam-]  aseI/asnI/vspI      bsaJI
                               nlaIII  alwI[dam-]  asp700        hhaI/cfoI nlaIII     mnlI     mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

      rsaI                                                                  nlaIV
      csp6I                                                               scrFI                      sfaNI
     nlaIV                                                                mvaI                           ppu10I
     kpnI                                                                 ecoRII                      nsiI/avaIII
     hgiCI                                                                dsaV                        nlaIII
     banI                             aluI                                bstNI                           sphI
     asp718    mnlI                  pvuII                                apyI[dcm+]                      nspI
     acc65I ddeI  aciI               nspBII                              bsaJI                            nspHI
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                              nlaIV
                    scrFI         scrFI
                    mvaI               mvaI                                ppu10I
                    ecoRII        ecoRII                            nsiI/avaIII
                    dsaV          dsaV                             nlaIII
                    bstNI              bstNI                              sphI
                    apyI[dcm+]         apyI[dcm+]                         nspI sfaNI
                   sexAI              bsaJI                               nspHI                        aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                               fnu4HI
                                                                                                               bglI
                                                                    nlaIII                                    sfiI
                                                                   styI                                       haeIII/palI
                                                                   ncoI                                     mnlI
                         aciI              bsrI               bslI dsaI                                 haeIII/palI
                aciI fokI              aciI       aciI        aciI bsaJI                           mnlI bsaJI aciI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAAAAAAAAT AAATACGTCT CCGGCTCCGG

                                                                                               scrFI
                                                                                               nciI
                                                                                               mspI
                                                                                               hpaII
                                                                                               dsaV
                                                            styI                             haeIII/palI
                                                            bsaJI                          mcrI
                                                            blnI                           eagI/xmaIII/eclXI
                                                         haeIII/palI           rmaI        eaeI
          haeIII/palI                                   stuI rmaI              maeI        cfrI
      mnlI      ddeI                      mnlI          haeI maeI             nheI        mspI cauII
      bsaJI mnlI   aluI                mnlI            mnlI avrII            aluI aluI    hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

           tfiI
           hinfI
        aciI                                                                                 fnu4HI
       thaI                                                       bstXI                      aciI
       fnuDII/mvnI                        aciI          scfI   sau96I   styI               thaI
       bstUI                pleI        rsaI         pleI      haeIII/palI                 fnuDII/mvnI  tru9I
       bsh1236I             hinfI       csp6I  scfI  hinfI     asuI      bsaJI             bstUI        mseI
                                                                                           bsh1236I   aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```

FIG. 10I

```
                  sau3AI
                  mboI/ndeII[dam-]
                  dpnI[dam+]
                  dpnII[dam-]
                  alwI[dam-]
                taqI[dam-]
              claI/bsp106[dam-]
             sau3AI
             mboI/ndeII[dam-]
             dpnI[dam+]
             dpnII[dam-]                                                                sau96I
            alwI[dam-]                                                                  avaII
                                                                                        asuI
                                                                                     scrFI
                                                                                     mvaI
                                                                                     ecoRII                  rmaI
                                                                                     dsaV          thaI    maeI
                                                                                     bstNI         fnuDII/mvnI
                                                                                     apyI[dcm+]          bstUI  nheI
                                 fokI                                        bslI  bsaJI    mnlI   bsh1236I  aluI
                                                                                            bsaJI  nruI   aluI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                            nlaIII
                           styI
                           pflMI
                           ncoI
          sfaNI     ecoRI  dsaI                                                   rsaI             aluI
       fnu4HI taqI  apoI   bslI fokI                      rmaI             gsuI/bpmI           pvuII tth111I/aspI
       bbvI claI/bsp106    bsaJI        nlaIII   fokI     maeI           bsrI   csp6I    ecoRV nspBII    bsrI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

        aluI
        sstI
        sacI
        hgiJII
        hgiAI/aspHI
        ecl136II
        bsp1286
        bsiHKAI
        bmyI                                  hphI
        banII            mnlI                 maeIII    bspMI                 taqI                        bsrI
      avaI           aciI                    bstEII   hphI        bsrI     hgaI          hphI   aluI   nlaIII
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```

FIG. 10J

```
                                                                                                  mspI
                                                                                                  hpaII
                                                                                                  bslI
                                                              gsuI/bpmI                           bsaWI
                                                             scrFI                        sau3AI
                                                             mvaI                                mboI/ndeII[dam-]
                                                     haeIII/palI                                 dpnI[dam+]
                                               fnu4HI      ecoRII                        dpnII[dam-]
           scrFI                                  aciI          dsaV                          alwI[dam-]
           mvaI                                thaI mnlI   bstNI                             nlaIV
           ecoRII                              fnuDII/mvnI apyI[dcm+]  pleI                  bstYI/xhoII
           dsaV                                    bstUI      rsaI   pleI gsuI/bpmI               bamHI
           bstNI      aluI                         bsh1236I   csp6I  hinfI     hinfI              alwI[dam-]
           apyI[dcm+]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT

                                                                                         sau3AI                  styI
                                                                                         mboI/ndeII[dam-]        bsaJI
                                                                                         dpnI[dam+]            rsaI
                                                                                         dpnII[dam-]           csp6I
                                                                                         alwI[dam-]           nlaIV
                                    fnu4HI  mboII                                       nlaIV                 kpnI
                                    bbvI    bpuAI                                       bstYI/xhoII           hgiCI
                                  scfI      bbsI                                        bamHI rsaI            banI
                                  pstI   mspI                                         mnlI    csp6I           asp718
                                  bsgI   hpaII                                  maeIII alwI[dam-]             acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

        sau3AI
        mboI/ndeII[dam-]                    mboII
        dpnI[dam+]          fnu4HI         bpuAI         aciI
        dpnII[dam-]         bbvI           bbsI    mboII                                  mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                                        scrFI
                                                                                        mvaI
                                                                                        ecoRII
                                                                                        dsaV
                                                                                        bstNI
                                haeIII/palI                                             apyI[dcm+]
      xmnI                     haeI     rsaI                   mnlI                               
      asp700                  mnlI     csp6I                   bslI         maeIII bsaJI        maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```

FIG. 10K

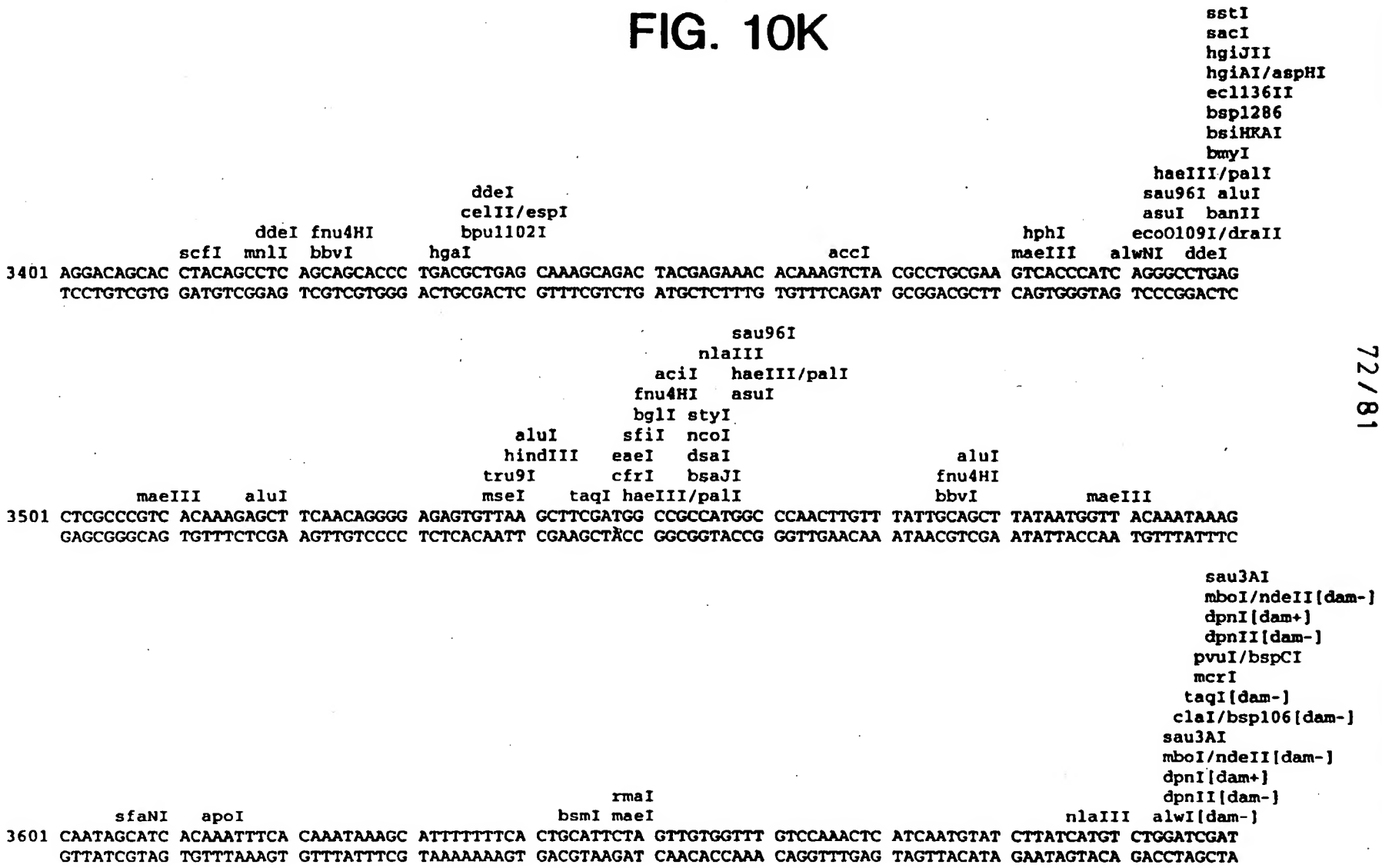

FIG. 10L

```
                                                                             rsaI
                                haeIII/palI                                   csp6I
                               haeI                                          nlaIV
          tru9I       fnu4HI styI                                            kpnI
          mseI        bbvI   ncoI                                            hgiCI
         aseI/asnI/vspI      dsaI                                            banI                        aluI
       xmnI         hinPI    bsaJI                                           asp718    mnlI              pvuII
       asp700       hhaI/cfoI nlaIII          mnlI       mnlI                acc65I  ddeI  aciI          nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                                nlaIV                                                                         nlaIV
                             scrFI                           sfaNI                 scrFI        scrFI
                             mvaI                               ppu10I                   mvaI                 mvaI
                             ecoRII                        nsiI/avaIII             ecoRII       ecoRII
                             dsaV                         nlaIII                   dsaV         dsaV
                             bstNI                            sphI                       bstNI                bstNI
                             apyI[dcm+]                       nspI                       apyI[dcm+]           apyI[dcm+]
                            bsaJI                             nspHI                     sexAI                bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                                  ppu10I
                                  nsiI/avaIII
                                 nlaIII
                                sphI
                                nspI sfaNI                                                                   bsrI
                                nspHI                                aciI         aciI fokI aciI            aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                                    fnu4HI
                                                                    bglI
                        nlaIII                                     sfiI
                       styI                                        haeIII/palI
                       ncoI                                      mnlI    mnlI      ddeI
                   bslI dsaI                                  haeIII/palI bsaJI mnlI  aluI                  mnlI
       aciI        aciI bsaJI                              mnlI bsaJI aciI   haeIII/palI                  mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```

FIG. 10M

```
              styI                                                                           scrFI
              bsaJI                                                                          mvaI
              blnI                                                                           ecoRII
            haeIII/palI               tru9I            haeIII/palI                           dsaV
           stuI rmaI                  mseI            eaeI                                   bstNI
           haeI maeI                 hpaI  aluI       cfrI                maeIII             apyI[dcm+]      tru9I
          mnlI avrII            aluI hincII/hindII  bsrI             maeII    bsrI          bsaJI  maeIII    mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                  sau3AI
                                                                  mboI/ndeII[dam-]
                                                         sau96I   dpnI[dam+]
                                                         haeIII/palI
                                         aluI          mnlI  aciI dpnII[dam-]
              fnu4HI                     pvuII       mboII asuI  pvuI/bspCI
              bbvI  fokI                 nspBII      earI/ksp632I mcrI                     bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

      hinPI
      hhaI/cfoI
     nlaIV                                                                        hinPI
     narI                                                                         hhaI/cfoI
     kasI                                                                        thaI
     hinlI/acyI                                                                  fnuDII/mvnI            hinPI
     hgiCI                                                                       bstUI          hinPI   hhaI/cfoI
     haeII      aciI                                                             bsh1236I       hhaI/cfoI
     banI   sfaNI                                                             rsaI      scfI fnu4HI   tru9I
     ahaII/bsaHI               sfaNI     aciI          aciI   maeII           csp6I     bslI  aciI    mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                        fnu4HI
        aciI           hinPI
      fnu4HI           hhaI/cfoI                       hinPI
      aciI            thaI                             hhaI/cfoI
     thaI             fnuDII/mvnI                     haeII
     fnuDII/mvnI      bstUI                     hinPI rmaI
     bstUI            bsh1236I     aciI         hhaI/cfoI    bsrBI
     bsh1236I     maeIII  bbvI maeIII           haeII  maeI  aciI        mboII              maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

FIG. 10N

```
                                   nlaIV
     mspI                         hgiJII                                              mnlI
     hpaII                        bsp1286                                           nlaIV
     naeI                         bmyI                                              hgiCI
     cfr10I           aluI        banII          nlaIV                              banI    taqI                    hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

        maeII     haeIII/palI
       draIII    sau96I                                  maeII   pleI          tru9I      pleI
       bsaAI     asuI                                    drdI    hinfI maeII   mseI       hinfI         bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                          thaI
                                                                                                          fnuDII/mvnI
                                                                                                          bstUI
                                                                            tru9I                         bsh1236I
         bslI                                                               mseI              tru9I     tru9I
        bslI   avaI                                          haeIII/palI           aluI      mseI  apoI mseI   apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                     hgiAI/aspHI
                                     bsp1286
                   maeII             bsiHKAI                                                                tth111I/aspI
                 tru9I               bmyI     ddeI                    aciI                                  maeIII
      tru9I      mseI                apaLI/snoI  rsaI               fnu4HI       tru9I                    maeII
      mseI     sspI  psp1406I        alw44I/snoI csp6I            sfaNI         mseI       aciI          bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                              hinPI
                                              hhaI/cfoI              mspI
                                             thaI                    hpaII
              hinPI                          fnuDII/mvnI            scrFI                                bsmAI
           fnu4HI                            bstUI                  nciI      aciI                      esp3I
           bbvI                       nspBII bsh1236I               dsaV sfaNI                         bslI
        nlaIII  hhaI/cfoI    aciI     aciI  hgaI       drdI         cauII fokI              aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                       thaI
                                                       fnuDII/mvnI
                                                       bstUI
                                                       bsh1236I
     scrFI                                            hinPI
     ncil                                             hhaI/cfoI                        mnlI
     mspI      nspI                                  thaI                          haeIII/palI
     hpaII fnu4HI                                    fnuDII/mvnI        mboII     sau96I
     dsaV aluI nspHI                                 bstUI mnlI         bpuAI     asuI
     cauII bbvI nlaIII   mnlI    hphI       hphI     bsh1236I           bbsI     eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                      nlaIV
                                                                     aciI
                                                                    thaI
                                                                    fnuDII/mvnI
                                  maeII                             bstUI
               nlaIII            hinlI/acyI                         bsh1236I
       tru9I rcaI                ahaII/bsaHI                       hinPI
       mseI  bspHI          ddeI aatII                             hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGGATAAACA AATAAAAAGA TTTATGTAAG

                  rcaI
                  bspHI
               bsrBI  bsmAI                                        mboII
               aciI nlaIII                          sspI           earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                        hgiAI/aspHI
                                                                                        bsp1286
                                                                               sau3AI   bsiHKAI
                                                                               mboI/ndeII[dam-]
                                                                               dpnI[dam+] bmyI
                                                                               dpnII[dam-]
             fnu4HI                                                      eco57I         apaLI/snoI
             aciI                     hphI           hphI            sfaNI mboII[dam-]  alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
             sau3AI
             mboI/ndeII[dam-]    sau3AI                                                                                  aciI
             dpnI[dam+]          mboI/ndeII[dam-]                                                                        thaI
             dpnII[dam-]         dpnI[dam+]                                                                              fnuDII/mvnI
            bstYI/xhoII          dpnII[dam-]                   psp1406I          hgiAI/aspHI                             bstUI
         bsrI         nspBII     alwI[dam-]                    xmnI              bsp1286  tru9I                          bsh1236I
     taqI   alwI[dam-]  aciI    bstYI/xhoII                    asp700            bsiHKAI  mseI                          hinPI
                                                          mboII maeII           bmyI    ahaIII/draI                     hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                scrFI
                nciI
                mspI
                hpaII
                dsaV
                cauII
              hinlI/acyI                                                                  rsaI
              hgaI                          aciI                                          csp6I  bsrI
              ahaII/bsaHI    bcgI    mcrI fnu4HI        ddeI                             scaI hphI    maeIII     sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                                            sau96I
                                                                                                            avaII
                                                                                                    sau3AI  asuI
                                                                                haeIII/palI         mboI/ndeII[dam-]
                                                                               eaeI                 dpnI[dam+]
                                                                               cfrI                 dpnII[dam-]
                                        fnu4HI                                 fnu4HI              pvuI/bspCI
      fokI  nlaIII                      bbvI          nlaIII                   aciI                mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                       nlaIII
                                   sau3AI maeIII                      mspI
                                   mboI/ndeII[dam-]    sau3AI   nlaIV
                                   dpnI[dam+]          mboI/ndeII[dam-]
                                  alwI[dam-]           dpnI[dam+]   hpaII
     aluI   aciI                nlaIII dpnII[dam-]     dpnII[dam-] bsaWI aluI                                  maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

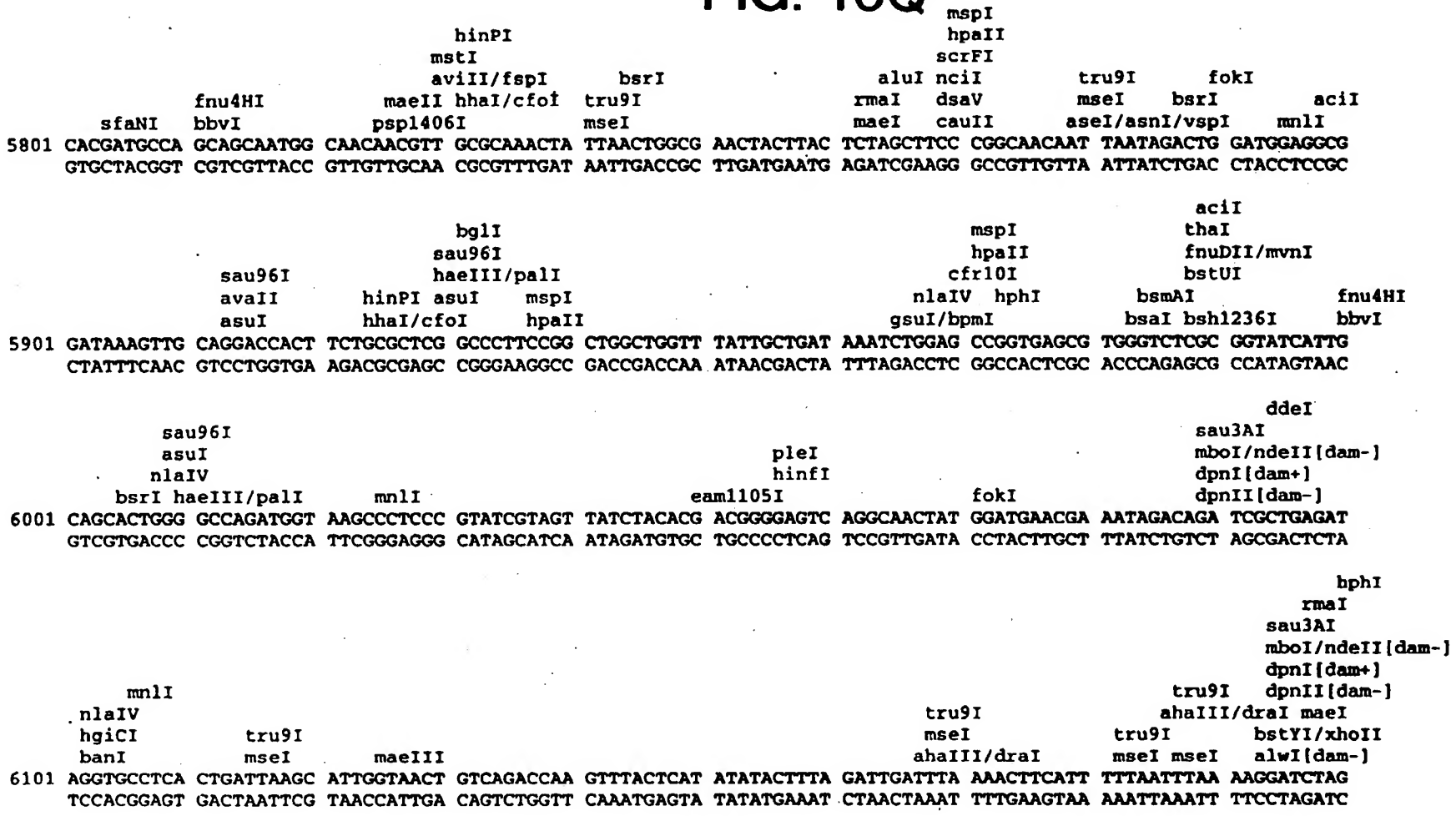

FIG. 10R

```
                                                                                                          sau3AI
       sau3AI                                                                                             mboI/ndeII[dam-]
       mboI/ndeII[dam-]                                                                                   dpnI[dam+]
       dpnI[dam+]                                                                                         dpnII[dam-]
       dpnII[dam-]                                                                                       bstYI/xhoII
       alwI[dam-]                                                                                 sau3AI  alwI[dam-]
      bstYI/xhoII        nlaIII             maeII                                                 mboI/ndeII[dam-]
     mboII[dam-]        rcaI            tru9I                                                     dpnI[dam+] mboII[dam-]
                        bspHI           mseI                          ddeI hgaI                   dpnII[dam-]
6201 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT
     CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA

                  thaI                                                                        sau3AI
     sau3AI       fnuDII/mvnI                                                                 mboI/ndeII[dam-]
     mboI/ndeII[dam-]                                                                         dpnI[dam+]
     dpnI[dam+]   bstUI                                                                       dpnII[dam-]
     dpnII[dam-]  bsh1236I                                                                   alwI[dam-]
     alwI[dam-]  hinPI      fnu4HI                                    aciI                  mspI
    bstYI/xhoII  hhaI/cfoI  bbvI                               aciI  nspBII                 hpaII     aluI
6301 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
     CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA

           bsrI         hinPI                      rmaI              haeIII/palI
        maeIII   eco57I     hhaI/cfoI                  maeI     bslI      haeI                       scfI      aciI
6401 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
     AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA

                                                                 scrFI
                                                                 nciI
                                      fnu4HI                     mspI                        mspI
                                alwNI  bbvI                      hpaII                       hpaII
                               bsrI  fnu4HI                      dsaV     pleI             bsaWI       hinPI
        mnlI                maeIII  bbvI      bsrI               cauII    hinfI          maeIII      hhaI/cfoI
6501 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG
     TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC

                                hgiAI/aspHI
        mcrI                    bsp1286
       aciI                     bsiHKAI
      nspBII                    bmyI                                                                       hinPI
     fnu4HI                     apaLI/snoI                                                                 hhaI/cfoI
     bbvI                       alw44I/snoI  aluI                         ddeI       scfI                 haeII
6601 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG
     GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC
```

FIG. 10S

```
                                                                                               scrFI
                                                                                               mvaI          scrFI
                                                                                               ecoRII  mvaI
                                             mspI                                              dsaV    ecoRII
                                             hpaII                                             bstNI         dsaV
                                             bslI    fnu4HI                  hinPI  mnlI       bsaJI         bstNI
                                aciI        bsaWI    aciI                    hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                 fnu4HI
                                                                                                                 aciI
                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                     taqI                           nlaIV                       bstUI
                                    mnlI  drdI    hgaI           sfaNI            aciI                          bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                         haeIII/palI
                      scrFI
                      mvaI
                      ecoRII
                      dsaV                    nlaIII
                      bstNI bslI                   nspI
        bslI          apyI[dcm+]      haeIII/palI  nspHI                      tfiI
      haeIII/palI nlaIV haeI        haeI           aflIII                     hinfI                    aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

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                                                                                                                   thaI
                                                                                                                   fnuDII/mvnI
                                                                                                                   bstUI
                                                                                                                   bsh1236I
                                                                                                                  hinPI
                                                                                                                  hhaI/cfoI
                                                                                                                 thaI
                                                                                        hinPI                    fnuDII/mvnI
                                 fnu4HI            fnu4HI                          sapI hhaI/cfoI                bstUI
                                 bbvI              bbvI   pleI                     mboII                         bsh1236I
                        bsrBI  aciI                hinPI  hinfI                    earI/ksp632I          mnlI    bslI
           aluI         aciI  fnu4HI      mcrI     hhaI/cfoI          mnlI    aciI    haeII         aciI         aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                      tru9I   aluI
        haeIII/palI           pvuII                                                     tru9I                    nlaIV
       eaeI tfiI   mseI       nspBII                                      hinPI         mseI      maeIII         hgiCI
       cfrI hinfI aseI/asnI/vspI                  bsrI       aciI         hhaI/cfoI  aseI/asnI/vspI mnlI         banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

        scrFI
        mvaI
        ecoRII
        dsaV
        bstNI
        apyI(dcm+)              mspI                          aciI                                               xmnI
       bsaJI                    hpaII                        bsrBI                            aluI      nlaIII   asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

      tru9I
      mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCI/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3	5,6, 9-12, 16-21
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search
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Date of mailing of the international search report
08.12.95

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Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	CELL, vol. 37, no. 3, July 1984 CELL PRESS,CAMBRIDGE,MA,US;, pages 1053-1062, C.L. CEPKO ET AL. 'Construction and applications of a highly transmissible murine retrovirus shuttle vector' cited in the application	1-3,7,8
Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21
Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21
Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21
Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21
A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21
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A	EP,A,0 260 148 (GENENTECH INC) 16 March 1988 see the whole document	1-21
A	EP,A,0 160 457 (GENENTECH INC) 6 November 1985 cited in the application see the whole document	1-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document	1-4

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/09576

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